# MTT SCIENCE 11

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## Doctoral Dissertation

Kaisa Kuoppala

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# Influence of harvesting strategy on nutrient supply and production of dairy cows consuming diets based on grass and red clover silage

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## Abstract

The main objective of this thesis was to elucidate the effects of regrowth grass silage and red clover silage on nutrient supply and milk production of dairy cows as compared with primary growth grass silages.

In the first experiment (publication I), two primary growth and four regrowth grass silages were harvested at two stages of growth. These six silages were fed to 24 lactating dairy cows with two levels of concentrate allowance. Silage intake and energy corrected milk yield (ECM) responses, and the range in these response variables between the diets, were smaller when regrowth silages rather than primary growth silages were fed. Milk production of dairy cows reflected the intake of metabolizable energy (ME), and no differences in the ME utilization were found between the diets based on silages harvested from primary growth and regrowth. The ECM response to increased concentrate allowance was, on average, greater when regrowth rather than primary growth silages were fed.

In the second experiment (publication II), two silages from primary growth and two from regrowth used in I were fed to rumen cannulated lactating dairy cows. Cows consumed less feed dry matter (DM), energy and protein, and produced less milk, when fed diets based on regrowth silages rather than primary growth silages. Lower milk production responses of regrowth grass silage diets were mainly due to the lower silage DM intake, and could not be accounted for by differences in energy or protein utilization. Regrowth grass silage intake was not limited due to neutral detergent fibre (NDF) digestion or rumen fill or passage kinetics. However, lower intake may be at least partly attributable to plant diseases such as leaf spot infections, dead deteriorating material or abundance of weeds, which are all higher in regrowth compared with primary growth, and increase with advancing regrowth.

In the third experiment (publications III and IV), red clover silages and grass silages harvested at two stages of growth, and a mixed diet of red clover and grass silages, were fed to five rumen cannulated lactating dairy cows. In spite of the lower average ME intake for red clover diets, the ECM production remained unchanged suggesting more efficient utilisation of ME for red clover diets compared with grass diets.

Intake of N, and omasal canal flows of total non-ammonia N (NAN), microbial and non-microbial NAN were higher for red clover than for grass silage diets, but were not affected by forage maturity. De-

laying the harvest tended to decrease DM intake of grass silage and increase that of red clover silage. The digestion rate of potentially digestible NDF was faster for red clover diets than for grass silage diets. Delaying the harvest decreased the digestion rate for grass but increased it for red clover silage diets.

The low intake of early-cut red clover silage could not be explained by silage digestibility, fermentation quality, or rumen fill but was most likely related to the nutritionally suboptimal diet composition because inclusion of moderate quality grass silage in mixed diet increased silage DM intake. Despite the higher total amino acid supply of cows fed red clover versus grass silage diets, further milk production responses on red clover diets were possibly compromised by an inadequate supply of methionine as evidenced by lower methionine concentration in the amino acid profile of omasal digesta and plasma.

Increasing the maturity of ensiled red clover does not seem to affect silage DM intake as consistently as that of grasses. The efficiency of N utilization for milk protein synthesis was lower for red clover diets than for grass diets. It was negatively related to diet crude protein concentration similarly to grass silage diets.

**Keywords:**
*grass silage, red clover silage, maturity, regrowth, milk production response, fibre kinetics*

# Timotei-nurminata- ja puna-apilasäilörehujen korjuustrategian vaikutus lypsylehmien ravintoaineiden saantiin ja maidontuotantoon

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## Tiivistelmä

Tämän väitöskirjatyön tarkoituksena oli selvittää eri kasvuasteilla korjattujen toisen sadon timotei-nurminatasäilörehun ja ensisadon puna-apilasäilörehun vaikutusta lypsylehmien rehujen syöntiin, ravintoaineiden saantiin ja maidontuotantoon. Vertailuruokinnoilla käytettiin ensisadon timotei-nurminatasäilörehuja.

Työhön sisältyi kolme koetta, joissa tehtiin yhteensä neljä ensisadon ja neljä jälkisadon timotei-nurminatasäilörehua ja kaksi ensisadon puna-apilasäilörehua kussakin yhteydessä kahdella kasvuasteella. Säilörehuja tutkittiin maidontuotantokokeessa ja kahdessa ravitsemusfysiologisessa kokeessa. Jälkisadon rehujen syönti ja lehmien maitotuotos sekä vasteiden vaihteluväli olivat pienemmät kuin ensikasvusta korjattujen rehujen. Maitotuotos heijasteli muuntokelpoisen energian (ME) saantia eikä energian tai valkuaisen hyväksikäytössä havaittu eroja eri satojen välillä.

Jälkisadon rehujen syöntiä ei rajoittanut kuidun sulatus, pötsin täyteisyys tai virtauskinetiikka. Pienempään syöntiin saattoi ainakin osittain olla syynä jälkisadossa runsaampana esiintyneet kasvitaudit, kuolleen kasvimassan osuus sekä rikkakasvit.

Kahdella eri kasvuasteella korjattuja puna-apilasäilörehuja verrattiin vastaaviin timotei-nurminatasäilörehuihin sekä näiden seokseen. Ravitsemusfysiologinen koe tehtiin viidellä lypsylehmällä. Huolimatta pienemmästä syönnistä ja ME-saannista maitotuotos oli puna-apilaruokinnoilla samalla tasolla kuin timotei-nurminataruokinnoilla. Tämä viittaa parempaan ME:n hyväksikäyttöön puna-apilasäilörehuja syöneillä lehmillä. Korjuuajankohdan myöhästyttäminen vähensi timoteinurminatasäilörehujen syöntiä, mutta näytti lisäävän sitä puna-apilaruokinnoilla.

Aikaisin korjatun puna-apilasäilörehun yllättävän pientä syöntiä ei selittänyt säilörehun sulavuus, käymislaatu, eikä pötsin täyteisyys, vaan se oli todennäköisimmin yhteydessä rehuannoksen ravintoaineiden epätasapainoon. Tähän viittaa se, että kun hyvin sulavaa, aikaisin korjattua puna-apilasäilörehua ja huonommin sulavaa, myöhään korjattua timotei-nurminatasäilörehua sekoitettiin, syönti lisääntyi selvästi. Vaikka puna-apilaruokinnoilla aminohappojen saanti oli runsaampaa kuin timotei-nurminataruokinnoilla, runsaampaa tuotosvastetta saattoi rajoittaa metioniiniaminohapon puute. Tähän viittaa metioniinin pienempi pitoisuus satakerran ruokasulassa ja plasmassa puna-apilaruokinnoilla.

Typen saanti ja virtaus pötsistä satakertaan olivat suurempia puna-apilaruokinnoilla verrattuna timotei-nurminataruokintoi-

hin. Typen hyväksikäyttö oli puna-apilaruokinnoilla heikompi kuin timotei-nurminataruokinnoilla, mutta se oli samalla tavalla kiinteästi yhteydessä rehuannoksen valkuaispitoisuuteen.

Tämän tutkimuksen tulosten perusteella voidaan suositella puna-apilan käytön lisäämistä säilörehun raaka-aineena. Toisen sadon timotei-nurminatasäilörehujen ominaisuudet olisi hyvä ottaa huomioon, kun säilörehueriä kohdennetaan eri eläinryhmien ruokintaan.

**Avainsanat:**
*timotei, nurminata, puna-apila, säilörehu, kasvuaste, toinen sato, maidon tuotanto, kuitukinetiikka*

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# Abbreviations

| | |
|---|---|
| AA | Amino acid |
| CP | Crude protein |
| DM | Dry matter |
| DMI | Dry matter intake |
| D-value | Concentration of digestible organic matter in dry matter |
| iNDF | Indigestible NDF |
| ME | Metabolizable energy |
| MP | Metabolizable protein |
| NDF | Neutral detergent fibre |
| NDS | Neutral detergent solubles |
| OM | Organic matter |
| OMD | Digestibility of organic matter |
| pdNDF | Potentially digestible NDF |
| SD | Standard deviation |

# List of original publications

This thesis is based on the following articles referred to in the text by their Roman numerals:

I Kuoppala, K., Rinne, M., Nousiainen, J. and Huhtanen, P. 2008. The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. Livestock Science 116: 171–182.

II Kuoppala, K., Rinne, M., Ahvenjärvi, S., Nousiainen, J. and Huhtanen P. 2010. The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows. Journal of Dairy Science 93: 3253–3263.

III Vanhatalo A., Kuoppala, K., Ahvenjärvi, S. and Rinne, M. 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. Journal of Dairy Science 92: 5620–5633.

IV Kuoppala, K., Ahvenjärvi, S., Rinne, M. and Vanhatalo, A. 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 2. Dry matter intake and cell wall digestion kinetics. Journal of Dairy Science 92: 5634–5644.



All experiments were conducted at MTT Agrifood Research Finland, Animal Production Research, in Jokioinen.

The author participated in planning and conducted with co-authors all the experiments and took full responsibility for the calculation of the results. The author was the main author for publications I, II and IV and participated as co-author in preparation of the publication III.

# Contents

# 1 Introduction

A significant proportion of the income in agriculture originates from milk and meat production based on ruminant animals. In Finland it was 48% of total agricultural income in 2008 (Statistics Finland 2010). Grassland products (silage, hay, pasture) constituted 54% of the feed energy units consumed by dairy cows in Finland in 2009 (ProAgria 2010). Therefore, the information about nutritive value and quality of forage is essential.

Boreal climatic conditions are characterised by a short growing period, which prevents continuous regrowth of leys. Also the seasonal variation is large. In Finland, the growing season is very short, even in Southern parts of the country, and development and growth of plants is fast, leading to considerable changes in morphological and chemical composition, and digestibility of herbage. At the same time, with increasing yield of feed dry matter (DM), the digestibility decreases. The effect of the timing of first harvest in early summer on the digestibility and intake of feeds, and subsequently the nutrient supply and milk production, is well documented (Rinne 2000). However, the production responses to varying quality of regrowth silages have not been investigated widely. In the future, climate change will lengthen the thermal growing season (Peltonen-Sainio et al. 2009). This will enhance the importance of regrowth harvest, which may increase and be challenged by increased occurrence of plant diseases and pests.

The proportion of regrowth in the total production of herbages for silage cannot be separated from crop production statistics, but one approach to estimate the usage of it is from number of silage samples from Finnish farms analyzed during the recent indoor feeding period (Nousiainen, J., Valio Ltd, personal communication). The number of samples from regrowth (cut 2 or cut 3) silages was approximately 43% of the total farm samples analyzed. Assuming that the number of samples taken from all cuts is relative to production of different silages, the amount of silage produced from regrowths could be calculated to be approximately 3400 million kg per year (Tike 2010).

Milk production has been lower when regrowth grass silages rather than primary growth grass silages were fed (Castle and Watson, 1970; Peoples and Gordon, 1989; Heikkilä et al. 1998; Khalili et al. 2005). Intake of regrowth silages was often lower in these studies. However, in many experiments, comparing primary growth with subsequent regrowths (second or third cut), only one silage per cut was used, leading to possible interactions between the harvest and digestibility, DM and neutral detergent fibre (NDF) concentrations, or fermentation quality of the silages. Because of these confounding factors, the reasons for the lower milk yield and intake potential of silages harvested from regrowth versus primary growth grass have remained unclear.

The Finnish studies concerning harvesting time of forages have been conducted with grasses, mainly timothy and meadow fescue. Red clover, the most important forage legume in Finland, has been studied considerably less. It differs from grass species, like other legumes, both in terms of chemical composition, and feeding value characteristics (Van Soest, 1994). Red clover plays an increasingly significant role in future silage production owing to its $N_2$ fixing ability. Utilization of this atmospheric N in terms of silage production has become attractive, not only for organic farmers, but also for conventional farmers, since intensive grassland production is largely dependent on fossil fuel based

N fertilizers, the costs of which have been high. According to an economic study of Doyle and Topp (2004), red clover can produce higher profits per hectare, compared with grass-based systems using high levels of N fertilizer.

Red clover is usually grown as a mixture with grass species with variable proportions of red clover. The proportion of red clover in mixed swards used in practical farming is difficult to measure, and is not reported in the statistics of arable land usage (Tike 2010). According to a survey including 355 farms, 35% of farmers declared that they use, or had used red clover, mainly for silage (Pursiainen et al. 2007). One way to estimate the proportion of red clover in mixed silages is to use concentration of Ca, since this is higher for red clover than for grasses (Rinne et al. 2010). According to the concentration of Ca in farm silage samples (Artturi 2010), the proportion of red clover containing silages samples was 18%. A much lower value of 7.5% was obtained from the number of farm silage samples from the recent indoor feeding period.

Most of the earlier research conducted in Finland has been made using mixed grass red clover swards, where the proportion of red clover has changed from 30 to 75 percent (Heikkilä et al. 1992, 1996). Comparisons between plant species are complicated because the feeding value of silage is also affected by maturity of the plants, preservation of silage and number of cut, which all cause variability between experiments. Further, effects of stage of maturity on red clover feeding value, intake or production responses have been studied much less than those of grass species. Thus, it is important to study the mechanisms of effect of red clover cultivated as a monoculture, although red clover is seldom fed as sole forage for dairy cows.

## *Objectives*

The objective of this thesis was to elucidate the effects of regrowth grass silage and red clover silage on nutrient supply of lactating dairy cows in milk production and physiological experiments. Diets based on grass silage from primary growth were used as controls. The experiments conducted were designed to answer following questions:

- Does the same digestibility in primary growth and in regrowth of grass silage affect the intake and production potential of cows in the same way?

- Does the change in digestibility of grass silage affect intake and production responses of cows similarly in regrowth and primary growth?

- Does the change in digestibility of silages affect intake and production of cows similarly in red clover silage compared with grass silage?

- What reasons may be found for inferior intake of regrowth grass silage or for superior intake of red clover containing silage, compared with primary growth grass silage?

- Is the response of cows to concentrate inclusion independent of harvesting time or maturity of ensiled grass?

# 2 Material and methods

The work documented in the publications I–IV was conducted as three experiments (Table 1). The experimental procedures used are described in detail within individual papers (I–IV) and only a brief summary is presented here. A mixed ley of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) was harvested in Jokioinen, Finland (61 °N) at two stages of growth (early and late) in primary growth (cut 1) (Figure 1). These two areas were harvested at two stages of growth (early and late) during the regrowth (cut 2). These six silages were used in experiment 1 and four of them (two from primary growth and two from regrowth) were used in experiment 2. In experiment 3, silages were prepared from primary growth of mixed timothy meadow fescue, and pure red clover (*Trifolium pratense* var Jokioinen*)* stands and harvested at an early and a late stage of maturity. The swards were wilted slightly and preserved in bunker silos of 70 or 40 t capacity (grass silages from experiments 1, 2 and 3) and in clamps (red clover silages from experiment 3) with a formic-acid based additive applied at a rate of 5 to 5.4 or 6 l/t for grass and red clover, respectively.

Experiment 1 (I) was a milk production trial with 24 intact lactating Finnish Ayrshire cows. The experiment was conducted according to a cyclic change-over design and the dietary treatments were in a 6 × 2 factorial arrangement consisting of the six experimental silages (two primary growth and four regrowth silages) and two concentrate levels (8 and 12 kg/d) resulting in 8 observations per each diet. Experiment 2 (II) was a physiological trial where four silages of the six used in experiment 1 were fed to four cows fitted with rumen cannulae, with 8 kg/d concentrate. The study was designed as a 4 × 4 Latin Square. In experiment 3 (III, IV), red clover and grass silages, and a mixture of red clover and grass silages, were fed with 9 kg/d concentrates to five rumen cannulated cows. The study was designed as a 5 × 5 Latin Square. Experimental periods in each experiment lasted 21 days. The cows averaged 73 days in milk (SD 21.6), 620 kg liveweight (SD 66) and 32 kg (SD 3.50) milk yield in the beginning of the experiments.

Feed intake and milk yield were measured daily and recorded during the seven (I) or five (II, III, IV) last days of each period. Apparent in vivo digestibility of organic matter (OMD) of silages was measured with sheep by total faecal collection, and used to calculate the D-value of the silages (concentration of digestible OM, g/kg DM) and the concentration of metabolizable energy (ME). Apparent whole tract digestibility of the diets was determined in experiment 1 with 12 cows of high yielding block by using acid insoluble ash (AIA) as an internal marker, while in experiments 2 and 3 total collection of faeces was used.

Digesta flow entering the omasal canal was determined based on the composition of pooled spot samples, obtained by using the omasal sampling technique of Huhtanen et al. (1997) modified by Ahvenjärvi et al. (2000). Indigestible neutral detergent fibre (iNDF) and Yb-acetate were used as markers for the large particles and small particles, respectively, and LiCoEDTA (II) or Cr-EDTA (III, IV) was used as the liquid phase marker, in combination with the reconstitution technique (France and Siddons, 1986).

Rumen fill and kinetic parameters were determined with the rumen evacuation technique. Blood samples were taken in Exp. 3 from one coccygeal vessel considered to present arterial blood and one superficial epigastric vein considered to present venous blood.

Table 1. Description of the experiments.

| Art. | Exp | Animals | Design | Silages, cuts and growth stages | Measurements |
|---|---|---|---|---|---|
| I | 1 | 24 intact dairy cows | 6 × 2 factorial, cyclic change-over design | timothy-meadow fescue<br>- 2 silages from cut 1: early and late<br>- 4 silages from cut 2: early and late from regrowths of early and late cut 1<br>- 2 concentrate levels, 8 and 12 kg/d | Intake<br>Milk yield<br>Milk composition<br>Diet digestibility |
| II | 2 | 4 rumen fistulated dairy cows | 4 × 4 Latin Square | timothy-meadow fescue<br>- 2 silages from cut 1: early and late<br>- 2 silages from cut 2: early and late | Intake<br>Rumen contents<br>Milk yield and composition<br>Nutrient flow to omasal canal<br>Rumen and total digestibility<br>NDF kinetics |
| III IV | 3 | 5 rumen fistulated dairy cows | 5 × 5 Latin Square | - 2 timothy-meadow fescue silages in cut 1: early and late<br>- 2 red clover silages from cut 1: early and late<br>- mixed late grass and early red clover silage | Intake<br>Milk yield and composition<br>Rumen contents<br>Nutrient flow to omasal canal<br>Rumen and total digestibility<br>NDF kinetics |

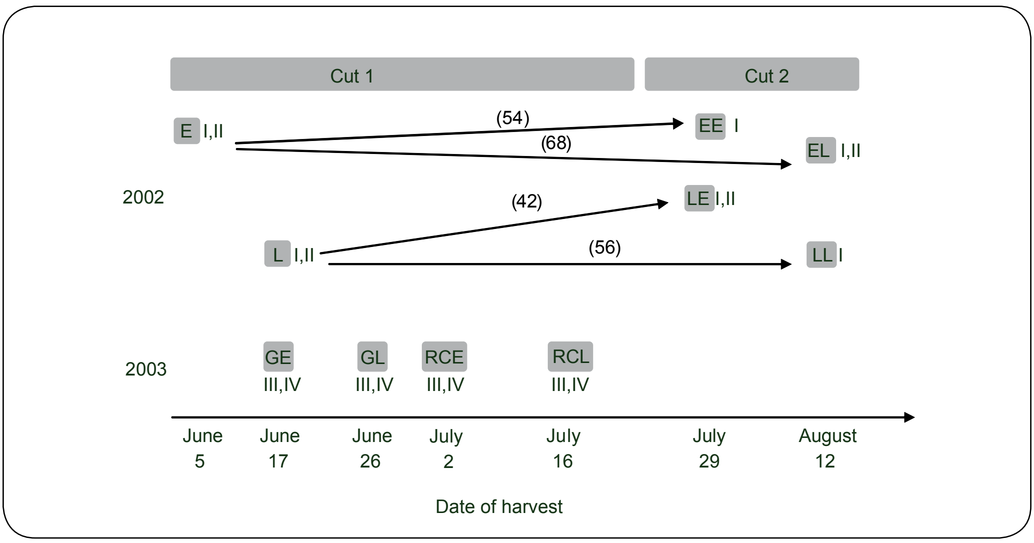


Figure 1. Schematic presentation of the harvesting strategies of grass silages in primary growth and in regrowth used in studies I and II and of grass (G) and red clover (RC) silages used in studies III and IV, harvested at early (E) or late (L) stages of growth in primary growth or at early (EE, LE) or late (EL, LL) stages of growth in regrowth. Growing days from cut 1 of each regrowth silage are shown in parenthesis.

# 3 Results and general discussion

## 3.1 Chemical composition and digestibility of forage

### 3.1.1 Red clover vs grasses

Legumes differ from grass species both in terms of chemical composition and feeding value characteristics (Van Soest 1994). In this work (III, IV), red clover contained more protein, ash and lignin and less NDF than grasses. This was in accordance with the average values collected from literature (Table 2). The collected data was primarily from experiments where red clover or regrowth grass was studied in the same experiment as grass from primary growth, which was used as a control.

The maturity effects of primary growth grass silages have been studied earlier (Rinne 2000), while the focus in this thesis is in regrowth grass and red clover. The higher ash content in red clover leads to a lower D-value compared with grasses at the same OMD. However, comparisons of forage digestibility are conducted in this thesis on the D-value basis, because ash contributes no energy to the animal.

Based on Table 2, the average concentration of neutral detergent fibre (NDF) was lower in red clover compared with grass, whereas the concentration of iNDF was higher, leading to a lower concentration of potentially digestible NDF (pdNDF) in red clover compared with grass. The mean iNDF concentration was 125 and 97 g/kg DM for red clover and grass, respectively. This is in accordance with recent data analyses, where the mean iNDF value of legume silages was 132 g/kg DM (Rinne et al. 2006b, data included red clover, lucerne and galega) and of grass silages 87 g/kg DM (Nousiainen et al. 2004).

The characteristics of NDF can be described by the ratio of iNDF to NDF. In IV, the ratio was 0.243 and 0.131 for red clover and grass silages, respectively, and in Table 2, the mean ratio was 0.350 for red clover and 0.180 for grasses. Higher ratios have been observed in legumes in many studies (Van Soest 1994, Huhtanen et al. 2006b), indicating different characteristics of cell wall in legumes compared with grasses. In legumes, the high lignin content is located entirely in the xylem where the concentration is such that the walls are completely indigestible, while other tissues are almost completely digestible (Wilson and Kennedy 1996). The lignin content of grasses is lower but it is distributed throughout all tissues except phloem, and the smaller concentration of indigestible material can therefore protect a larger amount of cell wall from digestion in grasses compared with legumes. It has been suggested that lignin and iNDF concentrations are linearly correlated (Smith et al. 1972, Van Soest et al. 2005), but it seems that lignin concentration cannot be applied universally to estimation of iNDF concentration or potential NDF digestibility (Huhtanen et al. 2006b).

The in-silo fermentation during silage preservation modifies the carbohydrate and nitrogenous fractions and both this and supplementary feeding affect how the silage intake potential is achieved in practical feeding situations (Huhtanen et al. 2007). The successful fermentation in silo depends on the characteristics of plant, preservation technique and weather conditions. Preservation of red clover and other legumes is more challenging compared with grasses because of the higher buffering capacity and lower concentration of water soluble carbohydrates (WSC) and DM (McDonald et al. 1991).

The lower sugar content of legumes (Table 2) was in accordance with McDonald et al. (1991). The average concentrations were 41 vs 66 g/kg DM for red clover and grass silages, respectively. The concentration of total fermentation acids was higher for red clover than for grasses. Red clover N was less degraded than grass N, as indicated by lower soluble N proportion in total N. Reduced degradation of N is at-

Table 2. Average chemical composition, digestibility and fermentation characteristics of grass and red clover herbage or silage in primary growth and regrowth (g/kg DM).

| | Grass | | | | | | Red clover | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Primary growth | | | Regrowth | | | Primary growth | | | Regrowth | | |
| | n | mean | SD | n | mean | SD | n | mean | SD | n | mean | SD |
| DM, g/kg | 35 | 288 | 90.8 | 37 | 283 | 92.9 | 15 | 253 | 77.8 | 17 | 281 | 67.7 |
| Ash | 44 | 78 | 17.3 | 41 | 93 | 12.4 | 21 | 96 | 9.9 | 17 | 100 | 12.6 |
| CP | 44 | 139 | 37.9 | 40 | 148 | 32.8 | 21 | 193 | 31.7 | 17 | 203 | 25.3 |
| NDF | 42 | 543 | 73.5 | 37 | 520 | 53.0 | 21 | 382 | 76.0 | 17 | 343 | 39.7 |
| iNDF | 19 | 82 | 37.8 | 17 | 84 | 34.2 | 8 | 129 | 51.2 | 9 | 116 | 38.9 |
| $iNDF_{lig}$ [1] | 10 | 114 | 60.0 | 12 | 147 | 83.3 | 2 | 152 | 77.7 | 3 | 191 | 44.3 |
| $iNDF_{omd}$ [2] | 12 | 88 | 28.7 | 12 | 79 | 29.8 | 6 | 99 | 40.4 | 4 | 114 | 6.3 |
| $iNDF_{all}$ [3] | 41 | 92 | 43.0 | 41 | 101 | 59.3 | 16 | 120 | 51.8 | 16 | 130 | 44.7 |
| iNDF/NDF | 33 | 0.167 | 0.0680 | 36 | 0.193 | 0.0998 | 13 | 0.314 | 0.1500 | 14 | 0.386 | 0.1271 |
| pdNDF | 33 | 454 | 46.2 | 36 | 418 | 41.1 | 13 | 260 | 66.7 | 14 | 207 | 43.8 |
| Lignin | 19 | 34 | 18.2 | 17 | 43 | 22.4 | 9 | 41 | 20.4 | 7 | 51 | 26.4 |
| D-value | 37 | 672 | 41.3 | 37 | 655 | 46.0 | 15 | 637 | 45.0 | 15 | 630 | 31.5 |
| OMD | 38 | 731 | 53.5 | 38 | 725 | 58.0 | 15 | 703 | 50.4 | 15 | 699 | 36.1 |
| pH | 29 | 4.18 | 0.319 | 25 | 4.23 | 0.388 | 13 | 4.15 | 0.209 | 13 | 4.50 | 0.345 |
| WSC | 27 | 64.4 | 43.39 | 27 | 68.5 | 52.03 | 11 | 41.2 | 34.60 | 13 | 40.2 | 34.22 |
| Lactate | 29 | 48.1 | 21.50 | 25 | 52.3 | 28.68 | 13 | 56.0 | 27.04 | 13 | 57.4 | 28.29 |
| Acetate | 28 | 16.8 | 9.26 | 25 | 15.9 | 5.08 | 13 | 17.1 | 6.06 | 13 | 21.3 | 10.05 |
| Propionate | 18 | 1.13 | 3.397 | 16 | 0.59 | 1.155 | 9 | 0.20 | 0.252 | 6 | 0.24 | 0.262 |
| Butyrate | 29 | 0.61 | 0.558 | 18 | 0.58 | 0.763 | 13 | 0.53 | 0.792 | 11 | 0.37 | 0.605 |
| Total acids | 29 | 65.7 | 30.35 | 25 | 69.0 | 31.26 | 13 | 73.8 | 32.53 | 13 | 79.2 | 37.05 |
| $NH_3$-N [4] | 28 | 54.4 | 20.54 | 28 | 75.3 | 61.19 | 13 | 54.9 | 24.44 | 12 | 61.6 | 25.09 |
| Soluble N [4] | 22 | 597 | 90.3 | 16 | 519 | 81.6 | 6 | 342 | 81.9 | 3 | 419 | 61.1 |

n = number of observations, SD = standard deviation, DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, iNDF = indigestible NDF, pdNDF = potentially digestible NDF, OMD = digestibility of organic matter, WSC = water soluble carbohydrates. [1]Concentration of iNDF was calculated with forage type specific lignin equations (Huhtanen et al. 2006b) when in situ estimates were not available. [2]Concentration of iNDF was calculated from OMD (Huhtanen et al. 2006b). [3]All iNDF determinations. [4]Expressed as g/kg total N. Data obtained from I, II, III, IV, Castle and Watson 1970, Gordon 1980, Åman and Lindgren 1983, Thomas et al. 1985, Peoples and Gordon 1989, Bosch and Bruining 1995, Huhtanen and Heikkilä 1996, Heikkilä et al. 1998, Jaakkola et al. 1999, Keady et al. 1999, Broderick et al. 2000, Fraser at al. 2000, Heikkilä et al. 2000, Khalili and Huhtanen 2000, Rinne and Nykänen 2000, Tuori et al. 2000, Ferris et al. 2001, Huhtanen et al. 2001, Heikkilä 2002a,b, Rinne et al. 2002, Tuori et al. 2002, Bertilson and Murphy 2003, Korhonen 2003, Khalili et al. 2005, Huhtanen et al. 2006b, Jaakkola et al. 2006, Rinne et al. 2006a, Ahvenjärvi et al. 2006, Broderick et al. 2007, Dohme et al. 2007, Hetta et al. 2007, Owens et al. 2008b, Pursiainen et al. 2008a, Pursiainen et al. 2008b, Vanhatalo et al. 2008, Moharrery et al. 2009, Kuoppala et al. 2010.

tributed to the endogenous presence and activity of polyphenol oxidase (PPO) in red clover inhibiting the proteolysis in the silo (Jones et al. 1995, Sullivan and Hatfield 2006). According to the fermentation characteristics the in-silo fermentation was somewhat more extensive for red clover silages than grass silages, but there were no indications of poor preservation.

### 3.1.2 Primary growth vs regrowth

During the growing period usually two or more cuts are harvested from the swards. In the current work, the first growth in spring is called the primary growth or first cut (cut 1). In southern Finland, the first cut is typically harvested in early to mid June for grass species, and somewhat later for red clover containing swards. After the first cut, the second cut (cut 2) is taken after circa 1 ½ months regrowth.

Regrowths of grass and red clover differ from primary growth with respect to tiller and chemical composition, digestibility and intake potential. Based on Table 2, the regrowths of grass and red clover contained more ash, CP, iNDF and less NDF compared with the primary growth of these forages. These average values give only a general view of the differences between cuts, as the variation in chemical composition is large within, and between the cuts, mainly due to the differences in harvesting time and environmental factors. The range of variation has often been larger in the first cut than in the second cut (Lindberg and Lindgren 1988, Huhtanen et al. 2006b, I).

The D-value was lower for regrowth grass compared with primary growth grass (Table 2). The same was true for Finnish farm silages, the mean for primary growth silages being 691 (n = 8310) and for regrowth 674 (n = 5530) g/kg DM in 2009 (Nousiainen, J., Valio Ltd, Finland, personal communication). For red clover containing farm silages these mean D-values were 673 (n = 573) and 662 (n = 457) g/kg DM, for cut 1 and cut 2, respectively. A higher iNDF concentration in regrowth with lower NDF concentration induced a higher ratio of iNDF to NDF for grass (0.167 and 0.193 for cut 1 and cut 2, respectively; Table 2), indicating a less favourable composition of fibre in regrowth grass silages.

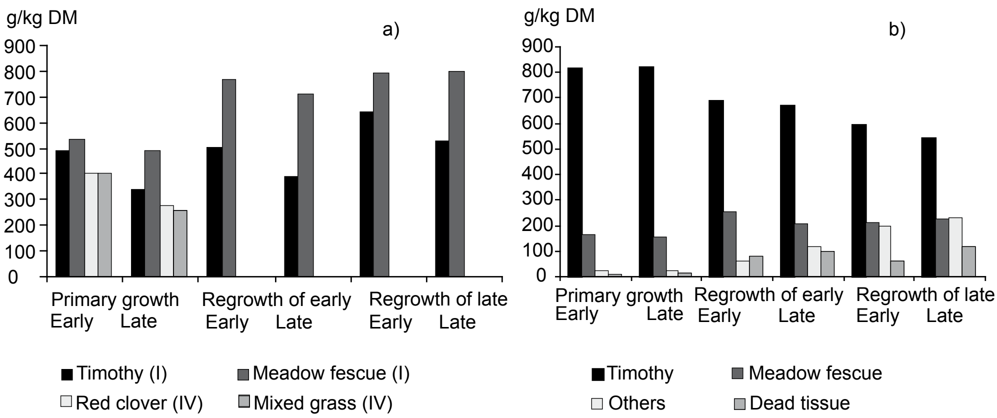


Figure 2. Proportion of leaves of timothy and meadow fescue in primary growth and regrowth (I) and of mixed grass (timothy and meadow fescue) and red clover in primary growth (IV) (a) and proportion of timothy, meadow fescue, other plant species and dead tissue in herbage (I)(b).

In regrowth, the proportion of leaves was higher than in primary growth (Figure 2a) in accordance with Fagerberg (1988) and Gustavsson and Martinsson (2004). Fagerberg (1988) reported that the phasic development in the regrowth period was different, so that the changes were slower, and the leaf stages had more leaves before the elongation phase began. In study I, meadow fescue was leafier in cut 2 than timothy, as was also reported by Åman and Lindgren (1983).

Higher proportions of other plant species (weeds) and dead tissues, and higher proportions of meadow fescue than timothy were found in regrowth, compared with primary growth, in study I (Figure 2b). In vitro digestibility of dead grass material has been reported to be very low and thus the high proportion of it may decrease the digestibility of whole herbage (Heikkilä et al. 2000, Pakarinen et al. 2008). Further, Heikkilä et al. (2000) analyzed the composition of brown dead leaves and found that concentrations of WSC, CP, potassium, and phosphorus were lower and that of ash, calcium and magnesium higher, compared with green leaves. Regrowth grass herbages also contain more waxes and cutins in cell soluble matter (Van Soest 1994), which have a low availability in animals and may cause the lower true digestibility of NDS for regrowth silages, compared with primary growth silages, as suggested by Huhtanen et al. (2006b).

For regrowth red clover, the mean values of ash, CP, iNDF and lignin were higher and concentration of NDF was lower, compared with primary growth (Table 2). The ratio of iNDF to NDF was higher in regrowth than in primary growth (0.386 and 0.314 for cut 2 and cut 1, respectively). Fagerberg (1988) reported that the regrowth gave a less clear picture of the development of red clover. Development was slower and the plants were in different developmental stages when the growth started after the first cut. Buds emerged from remaining stems and started flowering simultaneously as new stems developed. According to Frame et al. (1998) flowering in red clover does not appear to have a direct depressive effect on digestibility as it has in grasses. In mixed grass red clover sward the proportion of red clover has been reported to increase from cut 1 to subsequent regrowth (Randby 1992, Heikkilä et al. 1992, Søegaard and Weisbjerg 2007, Steinhamn and Thuen 2008).

### 3.1.3 Maturity in primary growth

With progressing plant growth, considerable changes occur. The effect of maturity has been investigated by delaying the harvest of forages, with the majority of research in this area focused on spring primary growth of grass. Typical effects of delayed harvest in primary growth are an increase of NDF and iNDF concentrations and a decrease of CP concentration and D-value (Rinne 2000, I, IV). Concentration of NDF is negatively correlated to OM digestibility, but NDF concentration can not be used as a predictor of OMD because NDF is not a nutritionally homogenic entity (Huhtanen et al. 2006b). It consists of potentially digestible parts (pdNDF) and totally indigestible parts (iNDF) and therefore, digestibility of NDF is also highly variable.

During the development of the plant, the ratio of stems to leaves increases, and the composition of stems changes, causing an increase of cell wall concentration at the expense of cell contents. The proportion of stem increases and the digestibility of stems decreases considerably, causing the major part of the decrease in whole plant digestibility (Brink and Fairbrother 1992, Kuoppala et al. 2010). Nordheim-Viken et al. (2009) reported that iNDF concentration of leaves did not change, whereas that of stems increased when timothy was harvested at three stages of growth. The iNDF concentration of the whole plant followed that of stems.

Morphological, anatomical and chemical changes and the intensity of change differ between plant species and the cut has considerable contribution to it. The mean daily change of D-value or chemical composition can be calculated from the results of the experiments with delayed harvest. These values tend to simplify the course of development, e.g. possible curvilinearity, but they are, however, a useful tool in describing and quantifying the phenomenon.

The average daily decrease of digestibility was collected from literature (Table 3). Digestibility was determined either in vivo or in vitro, for organic matter or dry matter. The mean daily change of digestibility was -5.3 (standard deviation (SD) ±1.6) g/d for grass silages and -3.7 (SD ±1.1) g/d for red clover in primary growth. The higher daily decline observed in IV for red clover, compared with grass, may stem from curvilinear development of the D-value found in the samples taken weekly from the same field during the growth of herbages used for these silages (Kuoppala et al. 2010). For these samples, the decline of D-value was very slow during the first four weeks (-0.8 g/d) and considerably faster during the next six weeks (-6.7 g/d), the silages of IV being prepared when the decline was steepest. A similar high daily decline was also reported by Hoffman et al. (1997) during year 2 but not year 1 (Table 3). These findings highlight the large variation between years which was also reported by Nordheim-Viken and Volden (2009).

The average daily rate of increase in NDF and iNDF concentrations in the Table 4 for primary growth grass silages was +4.6 (SD ±3.6) and +3.7 (SD ±1.2) g/d for grass silages, respectively, and +4.3 (SD ±3.1) and +4.1 (SD ±1.4) g/d for red clover silage, respectively. The increase of NDF in this work was much higher than those average values, the increase being +7.8–+8.0 g/d for grass (I, IV) and +6.3 g/d for red clover (IV). These high values for red clover NDF indicate curvilinearity as was also observed with the D-value in herbage samples of the same sward (Kuoppala et al. 2010). During the first four weeks of sampling, NDF concentration was decreasing and after that period it increased at a high rate of +8.6 g/d. Nordheim-Viken et al. (2009) reported an increase of +4.2 g/d in NDF concentration of timothy from the beginning of heading to full heading, but a decrease of -0.3 g/d from full heading to anthesis. While NDF concentration of timothy after heading remained quite constant, the concentration of iNDF increased, causing a decrease in pdNDF concentration (Nordheim-Viken and Volden 2009). Advancing maturity does not only increase the concentration of NDF, but also changes its composition and alters the rate of digestion of it.

### 3.1.4 Maturity in regrowth

The effects of maturity on digestibility of regrowth silages are quite variable in the literature. The average daily decrease of digestibility was -1.4 (SD ±1.5) g/d for grass silages and -1.6 (SD ±2.1) g/d for red clover in regrowth (Table 3). There may also be notable differences between grass species, and even varieties, in the level and rate of change of digestibility. The highest daily declines were observed with perennial ryegrass (Gordon 1980, Dawson et al. 2002) and lowest with timothy and meadow fescue (Åman and Lindgren 1983; Kuoppala et al. 2003, Rinne et al. 2007). However, the grass species were confounded with the geographical location because the data of perennial ryegrass was mainly from Great Britain and Ireland whereas the data of timothy and meadow fescue was from Finland and other Nordic countries (Table 3).

In many studies, the rate of decline of digestibility in regrowth grass has been very slow (Syrjälä and Ojala 1978, Van Soest et al. 1978, Åman and Lindgren 1983, Lindberg and Lindgren 1983, Keady and O'Kiely 1998, Kuoppala et al. 2003). The

Table 3. Daily change of digestibility (expressed as D-value (g/kg DM) or as digestibility of OM or DM, (g/kg)) of grass and red clover due to delaying the harvest.

| Reference | Method | Grass species[7] | Grass cut 1 | Grass cut 2 | Red clover cut 1 | Red clover cut 2 |
|---|---|---|---|---|---|---|
| Salo et al. 1975 | in vitro[1] | ti,mf | -6.7 | | -3.3 | |
| Syrjälä and Ojala, 1978 | in vivo | ti | -4.6 | -1.9[8] | | |
| Gordon, 1980 | in vivo | pr | | -3.9 | | |
| Steen and Gordon, 1980 | in vivo | pr | | -1.3 | | |
| Åman and Lindgren, 1983 | in vivo | ti | -2.9 | -1.9 | | |
| Åman and Lindgren, 1983 | in vivo | mf | -4.9 | -0.4 | | |
| Lindberg and Lindgren, 1988 | in vivo | ti | -4.3 | -1.2 | | |
| Tuori et al. 1992 | in vivo | mf | 5.9 | | | |
| Tuori et al. 1992 | in vivo | tf | 4.4 | | | |
| Hakkola and Nykänen-Kurki, 1994 | in vitro[2] | ti | -5.1 | | -1.7 | |
| Hoffman et al. 1997, year 1 | in vitro | | | | -2.7 | |
| Hoffman et al. 1997, year 2 | in vitro | | | | -4.9 | |
| Rinne et al. 1997 | in vitro[1] | ti,mf | -6.4 | | | |
| Keady and O'Kiely, 1998 | in vitro | pr | -4.7 | -1.2 | | |
| Rinne and Nykänen, 2000 | in vitro[3] | ti,mf | -5.7 | | -2.8 | -1.5 |
| Fraser et al. 2000 | in vivo | | | | | +1.8 |
| Dawson et al. 2002, year 1 | in vivo | pr | -3.7 | | | |
| Dawson et al. 2002, year 2 | in vivo | pr,rb | | -4.3 | | |
| Rinne et al. 2002 | in vivo | ti,mf | -4.8 | | | |
| Kuoppala et al. 2003 | in vitro[3] | ti | -5.5 | -0.6 | | |
| Kaldmäe et al. 2003, early varieties | in vitro[5] | | | | -5.7 | |
| Kaldmäe et al. 2003, late varieties | in vitro[5] | | | | -4.0 | |
| Kuoppala et al. 2006, trial 1 | in vivo | | | | -3.9 | -5.4 |
| Kuoppala et al. 2006, trial 2 | in vivo | | | | -4.8 | -1.4 |
| Kuoppala et al. 2006, trial 3 | in vivo | | | | -3.4 | -2.2 |
| Rinne et al. 2007 | NIRS[6] | ti,mf | -4.8 | +0.9 | -2.9 | +0.4 |
| Keady et al. 2008 | in vivo | pr | -3.5 | | | |
| Owens et al. 2008a | in vitro[1] | pr | | +0.4 | | |
| Owens et al. 2008b | in vitro[1] | pr | | +0.5 | | |
| Pursiainen et al. 2008a | in vitro[4] | ti,mf,pr | -10.3 | | | |
| Sihto and Rinne 2008 | in vitro[4] | ti,mf,tf | -4.5 | -1.9 | | |
| Vanhatalo et al. 2008 | in vivo | ti,mf | -5.1 | | -3.7 | -2.5 |
| Kuoppala et al. 2010 | in vitro[4] | | | | -3.7 | -1.6 |
| Nissinen et al. 2010 | in vitro[3] | ti | -8.4 | -0.9 | | |
| I | in vivo | ti,mf | -5.0 | -3.1 | | |
| IV | in vivo | ti,mf | -4.6 | | -4.9 | |
| **Mean** | | | **-5.3** | **-1.4** | **-3.7** | **-1.6** |
| Standard deviation | | | ±1.6 | ±1.5 | ±1.1 | ±2.1 |

Digestibility was determined in vivo with adult wethers or in vitro using methods of [1]Tilley and Terry (1963), [2]Menke et al. (1979), [3]Friedel (1990), [4]pepsine-HCl (Nousiainen et al. 2003), [5]DaisyII or using [6]near infrared reflectance spectroscopy (NIRS); [7]Plant species: ti = timothy (*Phleum pratense*); mf = meadow fescue (*Festuca pratensis*); pr = perennial ryegrass (*Lolium perenne*); rb = rough bluegrass (*Poa trivialis*); tf = tall fescue (*Festuca arundinacea*); [8]Third cut.

D-value has even increased in regrowth, with average daily increase of +0.9 g/d for timothy-meadow fescue, and +0.4 g/d for red clover in the field data of Rinne et al. (2007). Syrjälä et al. (1978) reported a decrease in OMD of -4.2 g/d in regrowth after the first cut but in the second regrowth, a curvilinear change was found, first an increase of OMD followed by a slight decrease. In general, the rate of decline has been slower in regrowth than in primary growth (Beever et al. 2000). This results in a smaller range in the quality of regrowth silages than in primary growth silages, although the time difference between the harvests within cuts was similar. Givens et al. (1993) reported considerably more variability in the values for the spring growths than in regrowth.

In study I, the increase in NDF concentration with delayed harvest in regrowth was marginal, but the increase in iNDF concentration was substantial, in accordance with Nordheim-Viken and Volden 2009. When the increase of iNDF was considered in relation to NDF accumulation (daily increase of iNDF/ daily increase of NDF), it was found to be much greater in regrowth than in primary growth (2.14 vs 0.48, respectively). In the Table 4, the average daily increases in NDF and iNDF concentrations were much lower for grasses than for red clover (+0.5 vs +3.1 g/d for NDF and +1.2 vs +4.0 g/d for iNDF, respectively). The digestibility of regrowth grass diets changed without any marked changes in NDF concentration (Figure 3), in accordance with Deinum et al. (1968) and Lindberg and Lindgren (1988). Lindberg and Lindgren (1988) reported only minor changes in NDF concentration with advancing maturity in regrowth with a slower rate of change than in primary growth. In the study I, the relationships between chemical composition and D-value differed between the cuts, as in primary growth similar D-value was achieved at a higher NDF concentration.

### 3.1.5 Environmental factors

Growth and change in chemical composition in plants are a complex process where the regulating mechanisms in the plant interact with environmental factors (Thorvaldsson 1987). Temperature, solar radiation, and water and nutrient availability are likely to contribute to growth and rate of cell wall lignification (Buxton and Fales 1994, Van Soest 1994). Furthermore, the time of the previous harvest has a remarkable effect on the regrowth of forage, and the annual and seasonal variation have also been considerable (Nordheim-Viken and Volden 2009).

Geographical location also alters forage quality even when forages are harvested at similar morphological stages (Buxton and Fales 1994). In the classical paper of Deinum et al. (1981), it was reported that digestibility of timothy was better at higher latitude. The environment alters leaf to stem ratios, causes morphological modifications and changes in chemical composition, and it also influences the rate of ageing and the amount of dead plant material (Buxton and Fales 1994).

Lower digestibility of regrowth silages observed in study I and in other studies (Castle and Watson 1970; Khalili et al. 2005)

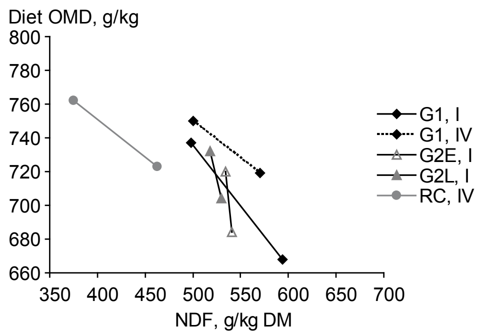


Figure 3. The relationship between the concentration of NDF in silage and the whole diet digestibility of OM (OMD) in dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2).

Table 4. Daily change of NDF and iNDF concentration of grass and red clover in primary growth and in regrowth due to delaying the harvest.

| | | NDF, g/d | | | | iNDF, g/d | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Grass | | Red clover | | Grass | | Red clover | |
| Reference | Grass species[1] | cut 1 | cut 2 | cut 1 | cut 2 | cut 1 | cut 2 | cut 1 | cut 2 |
| Åman and Lindgren 1983 | ti | +4.8 | -1.2 | | | | | | |
| Åman and Lindgren 1983 | mf | +4.6 | +1.3 | | | | | | |
| Lindberg and Lindgren 1988 | ti | +3.4 | -0.05 | | | | | | |
| Gasa et al. 1991[2] | pr | +4.3 | | | | +4.0 | | | |
| Tuori et al. 1992 | mf | +2.6 | | | | | | | |
| Tuori et al. 1992 | tf | +2.1 | | | | | | | |
| Brink and Fairbrother 1994 | | | | +2.4 | | | | | |
| Rinne et al. 1997 | ti+mf | +7.9 | | | | | | | |
| Fraser et al. 2000 | | | | | -0.8 | | | | |
| Rinne et al. 2002 | ti+mf | +7.6 | | | | +3.6 | | | |
| Dawson et al. 2002, year 1 | pr | +2.5 | | | | | | | |
| Dawson et al. 2002, year 2 | pr,rb | | +3.3 | | | | | | |
| Kuoppala et al. 2006 trial 1 | | | | +5.3 | +7.4 | | | +3.7 | +6.2 |
| Kuoppala et al. 2006 trial 2 | | | | +7.4 | +1.5 | | | +5.5 | +1.7 |
| Kuoppala et al. 2006 trial 3 | | | | -0.9 | +3.6 | | | +2.4 | +4.1 |
| Owens et al. 2008b | pr | | -1.1 | | | | +0.5 | | |
| Owens et al. 2008a | pr | | +0.4 | | | | +0.5 | | |
| Vanhatalo et al. 2008 | ti,mf | +4.0 | | 0.3 | 1.9 | | | | |
| Pursiainen et al. 2008a | ti,mf,pr | +14.0 | | | | +2.5 | | | |
| Keady et al. 2008 | pr | +4.6 | | | | | | | |
| Nordheim-Viken & Volden 2009[3] | | | | | | | | | |
| 59°N, year 1 | ti | +6.1 | +1.5 | | | +2.2 | +3.0 | | |
| 59°N, year 2 | ti | +2.8 | -0.3 | | | +6.1 | +1.2 | | |
| 59°N, year 3 | ti | +1.4 | +0.5 | | | +3.4 | +2.6 | | |
| 69°N, year 1 | ti | -2.0 | +0.4 | | | +4.9 | -0.1 | | |
| 69°N, year 2 | ti | -0.9 | +0.4 | | | +3.1 | +0.1 | | |
| Grabber 2009 | | | | +6.5 | +5.0 | | | | |
| Moharrery et al. 2009[4] | pr | +4.3 | +5.1 | +5.8 | +3.8 | | | | |
| Kuoppala et al. 2010 | | | | +3.7 | +1.6 | | | | |
| I | ti,mf | +8.0 | +0.7 | | | +3.9 | +1.5 | | |
| IV | ti,mf | +7.8 | | +6.3 | | +3.0 | | +4.9 | |
| **Mean** | | **+4.6** | **+0.8** | **+4.3** | **+3.2** | **+3.7** | **+1.2** | **+4.1** | **+4.0** |
| Standard deviation | | ±3.6 | ±1.7 | ±3.1 | ±2.6 | ±1.2 | ±1.1 | ±1.4 | ±2.3 |

[1]Plant species: ti = timothy (*Phleum pratense*); mf = meadow fescue (*Festuca pratensis*); pr = perennial ryegrass (*Lolium perenne*); rc = red clover (*Trifolium pratense*); rb = rough bluegrass (*Poa trivialis*); tf = tall fescue (*Festuca arundinacea*). The concentration of iNDF was determined by [2]4 or [3]6 d incubation while in other trials 12 d incubation time was used. [4]Cut 2 = third cut.

could be caused by the higher temperature during regrowth. The mean daily temperature reported in I was considerably higher for regrowth grass during summer (17.2 °C) than for primary growth grass during spring (12.0 °C), which may have affected the lignification process. Wilson et al. (1991) concluded that high temperature during growth increased the intensity of lignification of the existing lignified cells. High temperature promotes higher metabolic activity in plants and increases lignification (Van Soest 1994).

The concentration of lignin and iNDF of regrowth silages in study I was intermediate between the two primary growth silage. This is in accordance with the findings of Bertrand et al. (2008) who concluded that higher temperatures decreased DM and NDF in vitro digestibility, but had limited effect on concentrations of NDF, ADF and lignin.

Differences in the growing environment of primary growth versus regrowth also include a different relationship between the length of day and temperature. In spring, the correlation of the length of day and temperature is positive, but during regrowth it turns negative, i.e. days begin to shorten towards autumn. Deinum et al. (1981) concluded that each 1 h increase in day length increases herbage digestibility by about 0.02 units. However, Bertrand et al. (2008) did not find any difference between 15 h and 17 h photoperiods in the 17/5 °C temperature regime in DM or NDF in vitro digestibility. Nordheim-Viken et al. (2009) concluded from their experiment in a growing chamber that the temperature regime (21/15 °C and 15/9 °C, 12h/12h) or length of photoperiod (18 and 24 h) did not affect the iNDF or lignin content of timothy. Wilson et al. (1991) found, by using higher temperatures (32/26 and 22/16 °C, day/night), that OMD of leaf and stem was consistently lower at higher temperature. This difference was larger for grasses than for lucerne, and for stem it was twofold larger than for leaf.

## 3.2 Factors affecting intake of silages

Feed intake plays an essential role in production responses. Feed digestibility is one of the most important factors affecting silage intake (Huhtanen et al. 2007). Intake and digestibility depend on the rate of NDF digestion in the rumen, the rate of breakdown of feed particles and the rate of digesta outflow from the rumen (Mertens 1993). Most of the variation in diet digestibility in cows fed grass silage based diets was related to dietary NDF concentration and NDF digestibility (Nousiainen et al. 2009).

The intake of red clover containing silage has been superior (Heikkilä et al. 1992, 1996, Dewhurst et al. 2003a,b) but, in contrast, the intake of regrowth grass silage has been inferior (Castle and Watson, 1970, Peoples and Gordon 1989, Heikkilä et al. 1998, Khalili et al. 2005, I, II) when compared with grass silage prepared from primary growth. The reduced intake potential of regrowth grass silages clearly contributes to the lower milk production potential, but the challenge lies in identifying factors that cause the intake reduction.

Investigation of the effects of cut (primary growth vs regrowth) and maturity is not simple because these two factors are often confounded. For example, the low quality silage may have been cut late in primary growth and the high quality silage early in first or subsequent regrowth. In this situation, the difference between the harvests and the effect of maturity are confounded and conclusion drawn from those comparisons can be misleading.

### 3.2.1 Red clover vs grasses

The main findings in study IV concerning DMI of red clover vs grass were unexpectedly low DMI of early cut red clover silage and high DMI of the mixed red clover grass silage. The highest silage DMI was found for cows consuming the mixed forage diet, in accordance with previous physiological experiments with dairy cows (Dewhurst et al. 2003b, Bertilsson and Murphy 2003). However, Dewhurst et al. (2003a) reported higher intakes with dairy cows consuming red clover as sole forage, compared with mixed silage or grass silage.

In study III, mixing early cut red clover silage with late cut grass silage of lower digestibility caused a daily DMI increase of 2.0 kg, compared with the late cut grass silage diet, and as high as 2.7 kg when compared with early cut red clover diet. Mixing these two very different silages seemed to induce positive associative effects on intake. Intake increased in earlier experiments when the proportion of red clover increased from 0 to 67% (Tuori et al. 2002) or to 100% (Moorby et al. 2009) in the mixed red clover grass silage diets.

The mean silage DMI response to red clover containing silage was 1.3 (SD ±0.76) kg/d in data presented in Table 5. This is in good agreement with IV, and also with the relative silage DMI index which predicts higher intakes of silages containing legumes compared to pure grass silages (Huhtanen et al. 2007). In that meta analysis, the effect of D-value, DM, total fermentation acid and fibre concentrations were taken into account in the statistical model. The maximum increase of 1.65 kg/d for legume containing silages was reached at the inclusion rate of 0.80 of total silage DM. However, this value was based on limited amount of data so the equation was recommended for use when the proportion of red clover was less than 50% of the forage DM.

When red dover silage was fed as sole forage the silage DMI response was on average +0.4 (SD±2.0) kg/d, although the D-value was on average 40 g/kg DM lower (Table 6.) This increase was smaller and more varible compared with red clover-grass mixtures (Table 5) but the response in milk yield was higher. Thomas et al. (1985) reported a 21% increase in red clover silage intake over grass silage despite the lower digestibility. Broderick et al. (2001) compared red clover silage with alfalfa silage and cows consumed more red clover than alfalfa with no difference in milk production or composition. Replacing alfalfa silage with red clover silage increased intake and N efficiency but not milk yield (Broderick et al. 2007).

In study IV, the fermentation characteristics of the red clover and grass silages were fairly similar due to the high level of formic acid additive used for both silage types. A high D-value and low concentration of total fermentation acids generated the highest silage DMI index for early cut red clover silage, indicating that it should have promoted high DMI. The reason for the unexpectedly low DMI of highly digestible early cut red clover silage may be attributable to metabolic factors regulating feed DMI, because physical constraints could not be major restricting factors as will be discussed later. However, despite the lower DMI milk production was not decreased for red clover diets.

These findings suggest that, either early cut red clover satisfied the nutrient requirements for the milk yield potential of the cows used, or the nutrient composition was not optimal to reach higher DMI and milk production. The concentrations of VFA and ammonia in the rumen were highest on this diet, despite the lowest DMI (III). According to Choung et al. (1990), the intake of high-protein silages may be depressed by factors associated with high rates of absorption of ammonia from the rumen. However, the level of even the highest ammonia concentration peak during the feeding interval for the early cut red clover silage diet was low-

Table 5. Intake and production responses of dairy cows fed red clover containing silage compared with grass silage.

| | | Silage DMI, kg/d | Milk kg/d | ECM kg/d | Fat g/kg | Prot. g/kg | Prot. g/d | Diet OMD, g/kg |
|---|---|---|---|---|---|---|---|---|
| Heikkilä et al. 1992 | | | | | | | | |
| rc(30[1])+ti,mf[2] vs ti,mf | trial 1 | +1.7 | +1.6 | +1.0 | -2.1 | +0.1 | +58 | |
| rc(70)+ti,mf vs ti,mf | trial 2 | 0.0 | +1.1 | +0.1 | -2.4 | -1.4 | -2 | -30 |
| rc(70)+ti,mf vs ti,mf | trial 3 | +0.3 | -0.2 | -0.3 | -0.5 | +0.5 | +7 | -30 |
| Heikkilä et al. 2002b | | | | | | | | |
| rc(60)+ti,mf vs ti,mf | | +2.5 | +3.8 | +3.9 | +0.5 | -0.5 | +103 | -10 |
| rc(60)+ti,mf vs ar | | +1.6 | +0.4 | +1.0 | +2.2 | -1.2 | -25 | -70 |
| Tuori et al. 2002 | | | | | | | | |
| rc(33)+mf vs mf | | +2.3 | 0.0 | 0.0 | -0.1 | -0.1 | -3 | -10 |
| rc(67)+mf vs mf | | +1.6 | +0.5 | -1.0 | -4.1 | +0.2 | +19 | 16 |
| Tuori et al. 2000 | | | | | | | | |
| rc(50)+mf vs mf | | +0.2 | +1.3 | +0.8 | -0.7 | -0.4 | +25 | -1 |
| Bertilsson and Murphy 2003 | | | | | | | | |
| rc(50)+pr vs pr | year 1 | +1.9 | +1.6 | +0.6 | -3.2 | -0.5 | +40 | +27 |
| rc(50)+pr vs pr | year 2 | +0.8 | +1.3 | +0.6 | -2.0 | -1.2 | +10 | +18 |
| Dewhurst et al. 2003b | | | | | | | | |
| rc(50)+rg vs rg | year 1 | +1.5 | +3.7 | +4.3 | +1.5 | -0.5 | +113 | -33 |
| rc(50)+rg vs rg | year 2, 4 kg[3] | +0.5 | +0.2 | +0.2 | -0.6 | 0.0 | +19 | +35 |
| rc(50)+rg vs rg | year 2, 8 kg[3] | +0.9 | +1.1 | +0.2 | -3.1 | +0.7 | +55 | -17 |
| Vanhatalo et al. 2006 | | | | | | | | |
| rc(40)+mf,ti vs mf,ti | barley | +0.3 | +0.8 | +0.6 | -2.1 | +0.0 | +31 | +6 |
| rc(40)+mf,ti vs mf,ti | oats | 0.0 | +1.6 | +1.6 | +0.1 | -0.6 | +38 | -6 |
| Moorby et al. 2009 | | | | | | | | |
| rc(34)+pr vs pr | | +1.1 | +0.9 | +0.5 | -1.3 | -0.1 | +28 | -16 |
| rc(66)+pr vs pr | | +1.7 | +1.3 | +0.5 | -2.4 | -0.2 | +40 | -45 |
| III | | | | | | | | |
| rc(50)+ti,mf vs ti,mf | early | +0.8 | +0.7 | -0.2 | -2.3 | -1.1 | -9 | -19 |
| rc(50)+ti,mf vs ti,mf | late | +2.0 | +2.2 | +1.2 | -2.6 | -1.1 | +41 | +1 |
| **Mean** | | **+1.3** | **+1.3** | **+0.8** | **-1.4** | **-0.4** | **+31** | **-11** |
| Standard deviation | | ±0.76 | ±1.12 | ±1.45 | ±1.72 | ±0.63 | ±36.9 | ±26.8 |

DMI = dry matter intake; ECM = energy corrected milk; OMD = digestibility of organic matter; [1]Proportion of red clover in the mixture, %. [2]Plant species: ti = timothy (*Phleum pratense*); mf = meadow fescue (*Festuca pratensis*); pr = perennial ryegrass (*Lolium perenne*); rc = red clover (*Trifolium pratense*); rb = rough bluegrass (*Poa trivialis*); tf = tall fescue (*Festuca arundinacea*); rg = mixed ryegrass (*Lolium perenne*, *Lolium × boucheanum* and *Lolium multiflorum*). [3]Daily allowance of concentrates.

Table 6. Intake and production responses of dairy cows fed red clover silage as sole forage compared with grass silage.

| | | Silage DMI, kg /d | Milk kg/d | ECM kg/d | Fat g/kg | Prot. g/kg | Prot. g/d | Diet OMD, g/kg | Silage D-value, g/kg DM |
|---|---|---|---|---|---|---|---|---|---|
| Thomas et al. 1985 | | | | | | | | | |
| rc[1] vs. pr | | +1.8 | +2.0 | | -4.2 | -0.6 | +32 | -23 | -31 |
| Tuori et al. 2000 | | | | | | | | | |
| rc vs. mf | | +0.5 | +1.2 | +0.4 | -1.4 | -0.4 | +21 | +28 | -31 |
| Tuori et al. 2002 | | | | | | | | | |
| rc vs. mf | | -0.5 | +2.0 | +0.7 | -3.3 | -0.6 | +40 | +44 | -61 |
| Bertilsson & Murphy 2003 | | | | | | | | | |
| rc vs pr | year 1 | +0.9 | +2.3 | +1.6 | -1.7 | -0.5 | +60 | -50 | -25 |
| rc vs pr | year 2 | -2.2 | -0.4 | -2.0 | -2.5 | -2.2 | -70 | +4 | -31 |
| Dewhurst et al. 2003b | | | | | | | | | |
| rc vs. rg | year 1 | +2.0 | +3.2 | +3.3 | +0.7 | -1.2 | +75 | -71 | |
| rc vs. rg | year 2, 4 kg[2] | +2.6 | +2.1 | +2.7 | +1.8 | -0.4 | +53 | +14 | |
| rc vs. rg | year 2, 8 kg[2] | +2.6 | +2.7 | +1.2 | -3.6 | -0.7 | +62 | -6 | |
| Vanhatalo et al 2008 | | | | | | | | | |
| rc vs ti,mf | early cut | -3.6 | -0.3 | -0.7 | +0.3 | -1.5 | -52 | +86 | -57 |
| rc vs ti,mf | late cut | +0.7 | +2.9 | +2.0 | -3.1 | -1.0 | +72 | +113 | -28 |
| Moorby et al. 2009 | | | | | | | | | |
| rc vs pr | | +2.3 | +0.9 | -0.2 | -2.5 | -1.5 | -7 | -87 | |
| III | | | | | | | | | |
| rc vs ti,mf | early cut | -1.9 | +0.6 | -1.3 | -3.5 | -2.2 | -59 | +12 | -36 |
| rc vs ti,mf | late cut | +0.1 | +1.8 | +0.8 | -1.8 | -1.8 | +4 | +4 | -63 |
| **Mean** | | **+0.4** | **+1.6** | **+0.8** | **-1.9** | **-1.1** | **+18** | **+3.0** | **-40** |
| Standard deviation | | ±2.0 | ±1.2 | ±1.6 | ±1.8 | ±0.7 | ±51.0 | ±58.8 | ±15.8 |

DMI = dry matter intake; ECM = energy corrected milk; OMD = digestibility of organic matter; [1]Plant species: ti = timothy (*Phleum pratense*); mf = meadow fescue (*Festuca pratensis*); pr = perennial ryegrass (*Lolium perenne*); rc = red clover (*Trifolium pratense*); rb = rough bluegrass (*Poa trivialis*); tf = tall fescue (*Festuca arundinacea*); rg = mixed ryegrass (*Lolium perenne*, *Lolium* × *boucheanum* and *Lolium multiflorum*). [2] Daily allowance of concentrates.

er than the values of Choung et al. (1990) which reduced DMI.

Red clover silage has also proven to be superior in terms of feed intake and productivity for other ruminants than dairy cows: lambs offered red clover silage had higher silage and total DMI compared with lucerne or ryegrass silages resulting in a faster growth, increase in condition score and better feed conversion efficiency (Speijers et al. 2005). Merry et al. (2006) reported that steers consumed more red clover silage fed either as a mixture with grass, or as a sole forage, than grass silage.

### 3.2.2 Primary growth vs regrowth

In study I, silage and total DMI were higher when silages from primary growth rather than from regrowth were fed, but since the average silage D-value was also higher (674 vs 640 g/kg DM), this was to be expected. The response in silage DMI between diets containing grass silage from regrowth compared with primary growth grass silages, was quite systematically negative in Table 7. Huhtanen et al. (2007) reported, based on a meta-analysis of 46 diets, that the relative silage DMI index predicts lower intakes of regrowth grass silages compared with primary growth grass silages of similar digestibility, DM concentration and extent of in-silo fermentation. In the silage DMI index, the coefficient of the proportion of regrowth silage (0-1) was 0.44, meaning that it was on average 0.44 kg/d lower than that of comparable primary growth silage.

The lower DMI of regrowth silages was seen in study I when the mean of all four regrowth silages was compared to late harvested primary growth silage. Most of the chemical and digestibility parameters (for example, DM content 277 vs 283 g/kg and D-value 640 vs 644 g/kg DM, for regrowth silages and late primary growth silage, respectively) were similar and the only marked difference was the higher NDF concentration of primary growth silage. However, the cows fed silage from primary growth consumed more silage (13.3 vs 12.5 kg DM/d) and total DM and produced more milk (32.6 vs 31.1 kg ECM/d). It can be speculated that some other factors such as microbiological quality of silage possibly contributing to the taste or palatability in regrowth silages affect silage DMI.

The microbiological quality of regrowth grass may differ from that of primary growth, as typically weather is warmer later in the summer and regrowth grass contains more dead plant material. In study I, the occurrence of leaf spot infections (mainly *Drechslera* sp. and *Bipolaris sorokiniana*) was evaluated by sampling 6 times on a weekly basis both regrowth areas. The proportion of leaf area destroyed and the severity of damage increased with advancing regrowth (Figure 4). The 12 d difference in primary growth harvest did not affect systematically the occurrence of infections. Furthermore, the increased heterogeneity, in terms of higher proportion of weeds or different composition of grass species, in grass material may be one possible reason for the inferior intake of regrowth silages.

Mertens (1994) summarized that besides physical and metabolic regulation of intake, psychogenic modulators can affect intake. These modulators include taste,

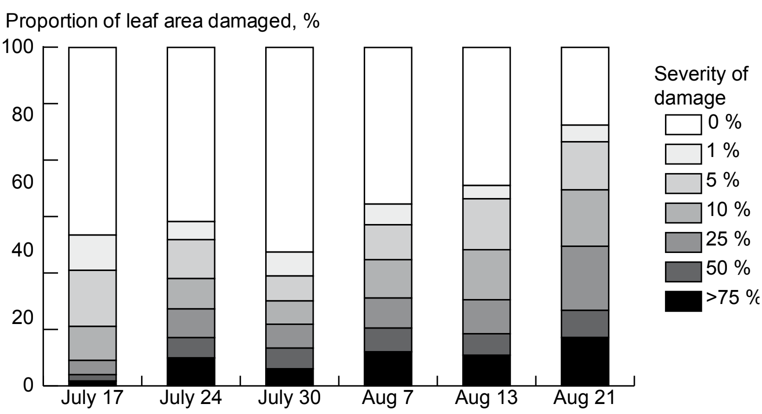


Figure 4. Average proportion of leaf area damaged by leaf spot infection (mainly *Drechslera* sp. and *Bipolaris sorokiniana*) during regrowth of grass cut at early or late stage of primary growth. (Parikka et al. unpublished data).

Table 7. Intake and production responses of dairy cows to regrowth grass and red clover silages.

| | | Silage DMI, kg/d | Milk kg/d | ECM kg/d | Fat g/kg | Prot. g/kg | Prot. g/d | Diet OMD g/kg | Silage D-value g/kg DM |
|---|---|---|---|---|---|---|---|---|---|
| Castle & Watson 1970 | | | | | | | | | |
| ti,pr[1] | | -0.8 | -0.4 | | -1.8 | +0.4 | -6 | | -13 |
| Peoples & Gordon 1989 | | | | | | | | | |
| pr | | -0.8 | -0.5 | | -2.1 | -0.5 | -24 | | -2 |
| Heikkilä et al. 1998 | | | | | | | | | |
| mf,ti | | -2.0 | -0.8 | -1.9 | -3.1 | -0.7 | -46 | +11.0 | +33 |
| Heikkilä 2000 | | | | | | | | | |
| cf | cut 2 vs cut 1 | -2.0 | -7.8 | -6.8 | +4.0 | +1.8 | -194 | | -84 |
| cf | cut 3 vs cut 1 | -2.4 | -7.4 | -5.0 | +6.3 | +3.2 | -147 | | -42 |
| Heikkilä 2002a | | | | | | | | | |
| ti | | -0.8 | -0.2 | -0.1 | +0.5 | -0.1 | -8 | | -35 |
| cg | | -2.0 | -1.3 | -1.6 | -0.4 | +0.2 | -37 | | -13 |
| Bertilsson & Murphy 2003[2] | | | | | | | | | |
| pr | | -2.0 | -2.0 | -3.7 | +3.4 | -2.2 | -120 | | -81 |
| Khalili et al. 2005 | | | | | | | | | |
| mf,ti | | -1.4 | -2.4 | -1.8 | +2.1 | -0.6 | -94 | -44 | -81 |
| Jaakkola et al. 2006 | | | | | | | | | |
| mf,ti | year 1 | -0.8 | -0.9 | -1.8 | -1.5 | -0.5 | -51 | -16 | -74 |
| mf,ti | year 2 | -2.6 | -2.8 | -4.1 | -2.0 | -1.2 | -133 | +3 | -69 |
| I | | | | | | | | | |
| ti,mf | conc.8, comp1 | -4.4 | -4.1 | -6.2 | -3.6 | -1.3 | -187 | -18 | -45 |
| ti,mf | conc.8, comp2 | -4.5 | -5.0 | -7.4 | -4.5 | -2.1 | -230 | -53 | -95 |
| ti,mf | conc.8, comp3 | -0.1 | +1.4 | +0.1 | -3.7 | -0.4 | +42 | +64 | +20 |
| ti,mf | conc.8, comp4 | -0.1 | -0.9 | -1.9 | -2.3 | -0.6 | -38 | +36 | -15 |
| ti,mf | conc.12, comp1 | -3.6 | -3.3 | -4.7 | -1.6 | -1.8 | -174 | +16 | -45 |
| ti,mf | conc.12, comp2 | -3.3 | -3.2 | -5.2 | -3.2 | -2.4 | -187 | -13 | -95 |
| ti,mf | conc.12, comp3 | -1.0 | +0.6 | 0.0 | -0.9 | -0.6 | +2 | +75 | +20 |
| ti,mf | conc.12, comp4 | -1.1 | -0.7 | -1.3 | -1.4 | -0.6 | -34 | +52 | -15 |
| **Mean, grass** | | **-1.8** | **-1.8** | **-2.7** | **-0.5** | **-0.6** | **-76** | **+11** | **-36** |
| Standard deviation | | ±1.3 | ±2.7 | ±2.7 | ±3.0 | ±1.3 | ±85.9 | ±38.0 | ±44.8 |
| | | | | | | | | | |
| Bertilsson & Murphy 2003 | | | | | | | | | |
| rc | | +3.3 | -0.1 | +0.4 | -0.3 | +0.5 | +10 | | -62 |
| Vanhatalo et al. 2008 | | | | | | | | | |
| rc | early | +2.1 | +0.8 | +0.5 | -2.0 | +1.2 | +61 | -77.0 | -20 |
| rc | late | +2.8 | +1.3 | +1.5 | +0.4 | +0.1 | +51 | -23.0 | 60 |
| **Mean, red clover** | | **+2.7** | **+0.7** | **+0.8** | **-0.6** | **+0.6** | **+40.7** | **-50.0** | **-7.3** |
| Standard deviation | | ±0.6 | ±0.7 | ±0.6 | ±1.2 | ±0.6 | ±27.0 | ±0.04 | ±63.6 |

DMI = dry matter intake; ECM = energy corrected milk; OMD = digestibility of organic matter; [1]Plant species: ti = timothy (*Phleum pratense*); mf = meadow fescue (*Festuca pratensis*); pr = perennial ryegrass (*Lolium perenne*); rc = red clover (*Trifolium pratense*); rb = rough bluegrass (*Poa trivialis*); tf = tall fescue (*Festuca arundinacea*); cf = cocksfoot (*Dactylis glomerata*); cg = couch grass (*Elymus repens*). [2]First and second cuts were harvested on different years.

smell, texture and visual appeal, in addition to emotional state, social interactions and learning. They involve an animal's behavioural response to inhibitory or stimulatory factors in the feed or feeding environment that are not related to the feed's energy value or filling effect (Mertens 1994). Maybe the taste or smell of regrowth grass deteriorated due to an increased amount of decomposing and infected leaf material and caused decreased intake. Also the increased proportion of weeds may have affected intake. If such factors exist in herbage, ensiling seems to maintain it.

Contrary to grass silages, higher mean silage DMI has been observed for regrowth red clover compared with primary growth (Table 7). However, the amount of data available was very limited. The two pair wise comparisons within the experiments of Bertilsson and Murphy (2003) and Vanhatalo et al. (2008) showed a high response of 2.7 kg/d to regrowth compared with primary growth red clover silages (Table 7). A higher intake of regrowth red clover containing silage compared with grass silage was observed also by Randby (1992).

### 3.2.3 Maturity

The average intake response to decreased grass silage digestibility in study I in primary growth (0.48 kg DM/d per 10 g decrease in D-value) was higher compared with the average value observed in the meta analysis (0.175, Huhtanen et al. 2007) (Table 8). Based on data of 17 publications, Rinne (2000) reported a decrease of 0.27 kg/d per 10 g decrease in D-value.

In regrowth (I), the average response was zero, when the change was considered between early and late harvesting in regrowth (Table 8). The lower DM concentration of earlier harvested regrowth silages may have affected the silage DMI response, as well as the more extensive in-silo fermentation (Huhtanen et al. 2007). Wilting, and consequently higher feed DM content, has been reported to improve intake of silage (Wright et al. 2000, Romney et al. 2000, Huhtanen et al. 2007). However, if we compare the diets based on the silages made from the regrowths of early harvested in cut 1 with those made of late harvested in cut 1, the 12-d longer growing time in cut 2 decreased the average D-value of silages (634 vs 647 g/kg DM), decreased silage DMI by 0.5 kg/d, the average response in silage DMI being –0.42 kg/d per 10 g decrease in D-value. In this comparison the responses were not confounded with DM concentration and in-silo fermentation of the silages.

Steen and Gordon (1980) and Gordon (1980) reported an average decrease of 0.24 kg/d per 10 g decrease in D-value. In physiological studies, the silage DMI responses were on the same level in primary growth and lower in regrowth (Table 8).

The tendency of increasing silage DMI with advancing maturity for red clover diet (IV) was opposite to the results found with grass silage diets in I, II and IV and in earlier studies (Rinne 2000). The delay of two weeks in the harvest of red clover caused an average increase of 0.8 kg in daily DMI despite the decreased digestibility, the increase being 0.12 kg/d per 10 g decrease in D-value (IV).

In contrast to IV, a two weeks' delay in the harvest of primary growth red clover silages resulted in a decrease of 1.7 kg in daily DMI in the experiment of Vanhatalo et al. (2008), the response of –0.36 kg/d per 10 g decrease in D-value being higher than the above mentioned values. Also Hoffman et al. (1997), who compared red clover with alfalfa silages, both harvested at two stages of growth in primary growth, found that delaying the harvest decreased silage DMI of both plant species.

Using regrowth red clover silages, Vanhatalo et al. (2008) found an increase of

1 kg in daily DMI when more mature red clover silage was fed. Fraser et al. (2000) also found in the experiment with lambs eating regrowth red clover silage that delaying the harvest did not affect the voluntary intake.

Although the results from dairy cows consuming red clover silage harvested at different growth stages are rare, and the effects of maturity may have been confounded with the cut and other quality components, increasing the maturity (ex-

Table 8. Change of silage DMI kg/d per 10 g decrease of digestibility of silage (expressed as D-value, OMD or DMD) due to delay in harvest.

| | Grass | | Red clover | |
|---|---|---|---|---|
| Reference | cut 1 | cut 2 | cut 1 | cut 2 |
| I, conc. 8 kg | -0.60 | | | |
| I, conc. 12 kg | -0.35 | | | |
| I, E, conc. 8 kg | | -0.02 | | |
| I, E, conc. 12 kg | | -0.06 | | |
| I, L, conc. 8 kg | | 0.00 | | |
| I, L, conc. 12 kg | | +0.03 | | |
| I, (EE&EL vs LE&LL), 8 kg | | -0.60 | | |
| I, (EE&EL vs LE&LL), 12 kg | | -0.24 | | |
| II | -0.28 | -0.11 | | |
| IV | -0.29 | | +0.12 | |
| Vanhatalo et al. 2008 | -0.78 | | -0.36 | +0.31 |
| Hoffman et al. 1997, year 1 | | | -0.78 | |
| Hoffman et al. 1997, year 2 | | | -0.33 | |
| Steen and Gordon 1980 | | -0.24 | | |
| Gordon 1980 | | -0.23 | | |
| Mean | -0.46 | -0.08 | -0.34 | +0.31 |

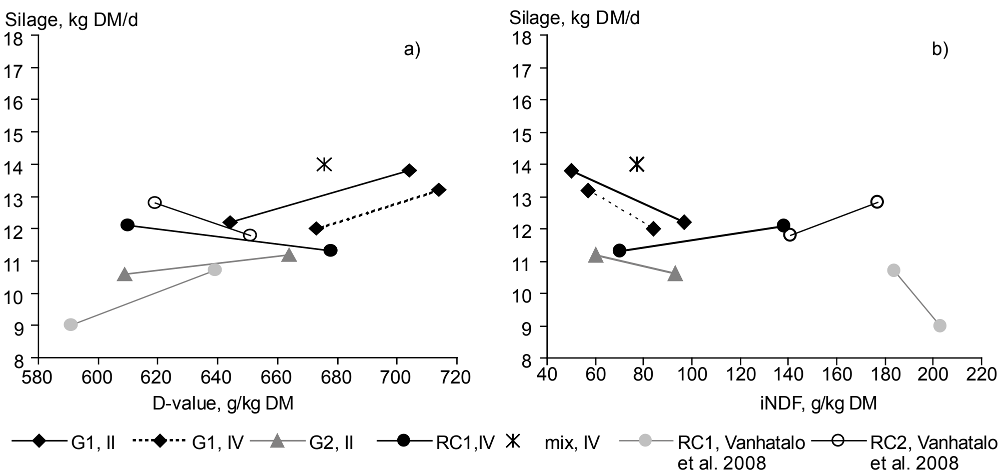


Figure 5. Silage dry matter intake in relation to silage D-value (a) and iNDF concentration (b) in dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2).

pressed as D-value) of the red clover ensiled did not seem to affect silage DMI as consistently as with grasses (Figure 5a). The intakes of all sets of grass silages and one set of red clover increased with increasing D-value, whereas the intake of two sets of red clover silages decreased.

The early harvested red clover used in III and the early harvested regrowth red clover in the experiment of Vanhatalo et al. (2008) were possibly harvested at a very early stage of maturity, resulting in increase in the DMI when harvesting was delayed. This was, however, not obvious, on the basis of the D-values of these silages. It seems that the D-value may not be as appropriate criteria for predicting the DMI response of red clover silages as it is for grass silages. However, the data available is not enough because stage of maturity of red clover used in the literature data could not be detected or connected to digestibility in majority of papers.

It can be speculated based on these data that silage iNDF concentration could be better in predicting the intake responses to red clover (Figure 5b). The DMI of grass silage decreased in relation to increasing iNDF concentration in silage. For red clover silage, the DMI first increased with increasing iNDF concentration and then decreased suggesting a curvilinear response to iNDF concentration of the silage.

Although general conclusion can not be drawn owing to the small amount of data available, the effect of maturity of red clover silage intake warrants for a further research. It would have been interesting to detect the intake responses to later stages of maturity for red clover.

## 3.3 Rumen contents and NDF digestion kinetics

Plant species and maturity are the two most important sources of variation in digestion kinetics (Smith et al. 1972, Mertens 1993). Physical distension of the reticulorumen is a major factor limiting intake of high fibre diets (Mertens 1994). This means that in the short term regulation of intake, stretch receptors in the reticulorumen provide the stimulus to the brain which ends the meal. When high quality diets like highly digestible silage are fed, the cow eats to meet its energy demand and DMI is limited by the cow's genetic potential (Mertens 1994).

Data concerning rumen fill and digestion and passage kinetics of regrowth grass compared with primary growth is very limited, whereas the effect of maturity in primary growth has been studied more. In study II, no difference between cuts were observed for rumen volume or pool sizes of fresh matter, DM and NDF.

Volume and fresh weight of rumen contents were higher for the late harvested red clover and for the mixed red clover grass diet compared with grass diets (IV) and compared with grass silage diets in II. Rumen pool sizes of NDF and pdNDF were smaller for red clover silage diets than grass silage diets (Figure 6a, b), in accordance with Dewhurst et al. (2003a). In contrast, the pool sizes of iNDF for both red clover diets were larger than for the grass diets (Figure 6c, d). The same was true for regrowth grass silage diets compared with primary growth grass silage diets (II)

The larger pool size of iNDF observed both for red clover and regrowth grass diets was due to the slower iNDF outflow rate (II, IV), which also resulted in a higher ratio of iNDF to pdNDF in the rumen contents, compared with primary growth grass diets. This ratio was higher in the omasal canal for grass diets, indicating selective retention of highly digestible particles. The opposite was true for red clover diets. It seemed that iNDF accumulated into the rumen with red clover diets, but it is difficult to draw conclusion about cause and effects: is it due to plant characteristics or is it because rumen fill, in terms of NDF pool, was less limiting with red clover diets so that there has not been a need for faster passage?

The pool size of pdNDF was higher for primary growth grass diets than for diets based on regrowth grass (II) or red clover silages (IV). The higher digestion rate of pdNDF for red clover diets resulted in a smaller pool size of pdNDF and NDF, compared with grass silage diets (IV). It has also been reported in many other studies that legumes have a higher digestion rate compared with grasses despite the higher content of indigestible material (Smith et al. 1972, Wilson and Kennedy 1996, Weisbjerg and Søegaard 2008). Further, Rinne and Nykänen (2000) reported that the difference in digestion rate between red clover and timothy was much higher for stems than leaves.

Regardless of the higher passage rate from rumen for red clover pdNDF, the higher digestion rate of red clover pdNDF relative to grass silage diets besides lower pdNDF concentration resulted in a higher total tract pdNDF digestibility for red clover silage diets. Similar total tract NDF digestibility in combination with a higher concentration of cell solubles for red clover diets resulted in a similar total tract OM digestibility of these diets. In Table 6, av-

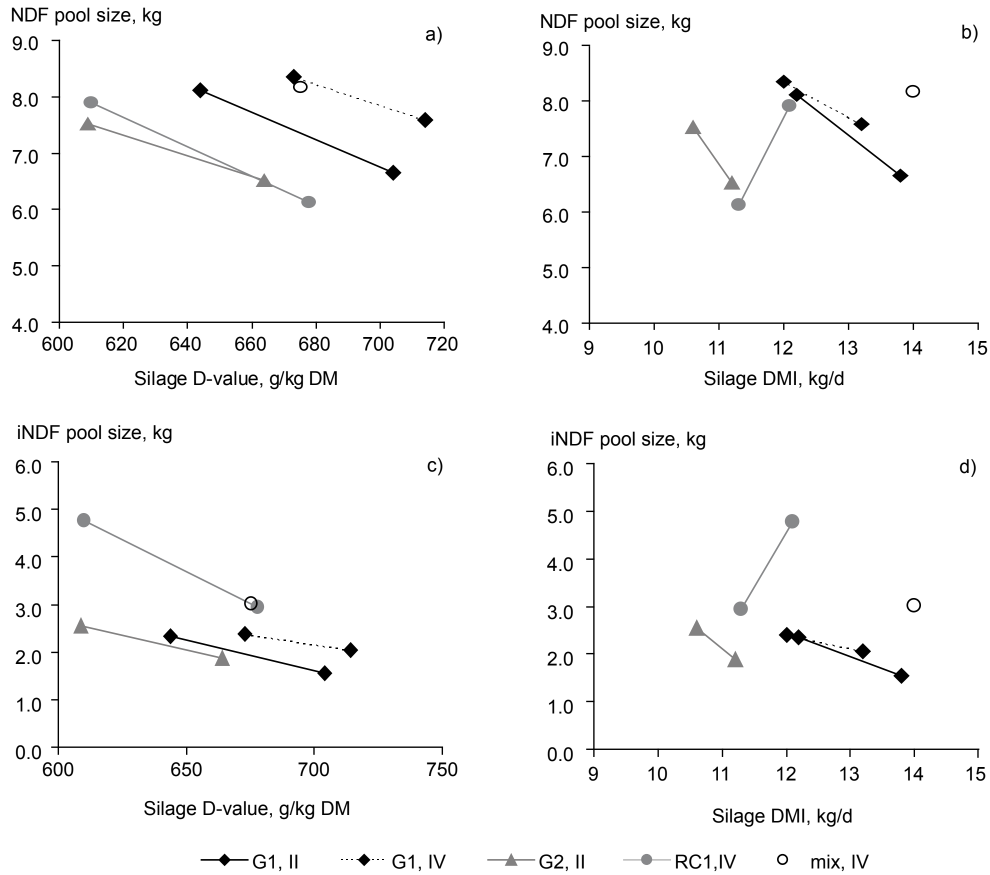


Figure 6. The effect of silage D-value (a, c) and silage DMI (b, d) on the rumen pool size of NDF and iNDF in studies II and IV for dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2).

erage diet OMD was on the same level for pure red clover diets compared with grass silage diets.

Delaying the harvest increased the pool sizes of NDF, pdNDF and iNDF in both cuts in II (Figure 6), which was in accordance with earlier results in primary growth (Rinne et al. 2002). Delaying the harvest decreased the digestion rate of pdNDF for grass (II, IV) but increased that for red clover in study IV (Figure 7). It did not affect passage rate of iNDF (II, IV) but increased that of pdNDF (II). Advancing maturity has been shown to decrease the digestion rate for both legumes and grasses (Buxton 1989, Rinne and Nykänen 2000) but the change was slower for red clover than for grasses.

Hoffman et al. (1997) found a faster in vitro NDF digestion rate for later harvested legumes for the first year, but not for the second year. Moharrery et al. (2009) observed no clear effect of maturity on in vitro fractional rate of degradation of NDF for red clover or grasses although the later harvest in primary growth seemed to decrease degradation rate for grasses. Rinne and Nykänen (2000) reported that the digestion rate for the leaves of red clover and grass (timothy) was almost the same, while for stems of red clover it was clearly higher than for the stems of timothy. The increasing proportion of stems with advancing maturity did not appear to affect the digestion rate for red clover as negatively as for grasses.

The total tract digestibility of pdNDF for both grass and red clover diets did not de-

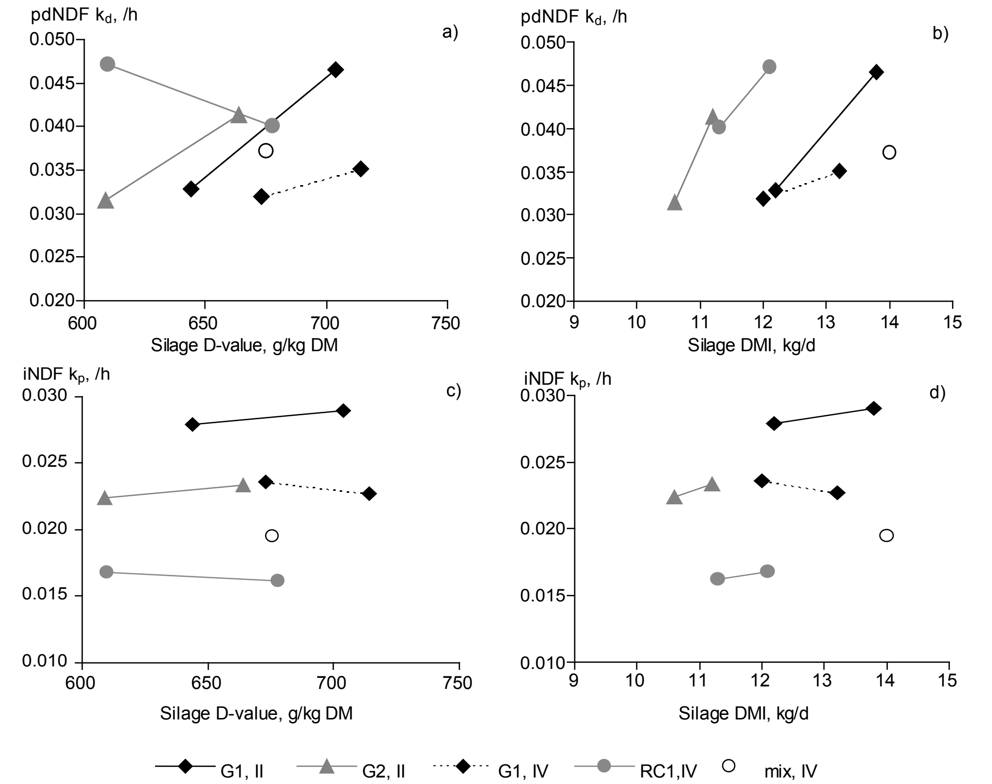


Figure 7. The effect of silage D-value (a, c) and DM intake (DMI) (b, d) on digestion rate ($k_d$) of pdNDF and passage rate ($k_p$) of iNDF in dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2).

crease with the delay in harvest in study IV. It can be speculated that the different responses to the delay of harvest found for red clover diets (seen in Figures 6 and 7) may originate from the fact that cows consumed more red clover silage harvested at a later stage of growth. Mertens (1994) stated that the level of intake has the greatest impact on the rate of passage and it is not clear whether an increased rate of passage results in increased intake or increased intake causes increased rate of passage. According to Huhtanen et al. (2006a) the passage kinetics often reflect the effects of feed intake, which may be the case in the present study, where the DMI was increased by delaying the harvest for red clover.

Rumen fill has most probably not limited intake of regrowth grass silages (II) because the highest rumen pool size of NDF was observed for late harvested primary growth silage. Intake of NDF was also highest for that diet. Declining NDF intake with high silage D-values (over 640 g/kg DM) suggest that factors other than rumen fill were limiting intake (Huhtanen et al. 2007).

The unexpectedly low DMI of early cut red clover silage (IV) could not be attributable to physical constraints because rumen pool sizes of DM and NDF were smallest. Rumen volume and pool sizes of fresh matter, DM and NDF were higher for mixed diet than for late cut grass, or early cut red clover silages fed alone, which is in accordance with Bertilsson and Murphy (2003). Dewhurst et al. (2003) found larger pool sizes for red clover fed as sole forage compared with 1:1 mixture or grass silage alone. The difference between the present experiment and those two may stem from different cuts: Bertilsson and Murphy (2003) had their silages made from cut 2 and the silages of Dewhurst et al. (2003) were proportional mixtures of 3 cuts of each year, while in IV, primary growth silages were used.

Rumen fill may have limited DMI of late harvested primary growth grass silage where the rumen content of NDF was highest in II and IV. In IV, cows consumed 2 kg/d more silage DM when early harvested red clover was mixed with late harvested primary growth grass silage with a decrease in NDF pool size.

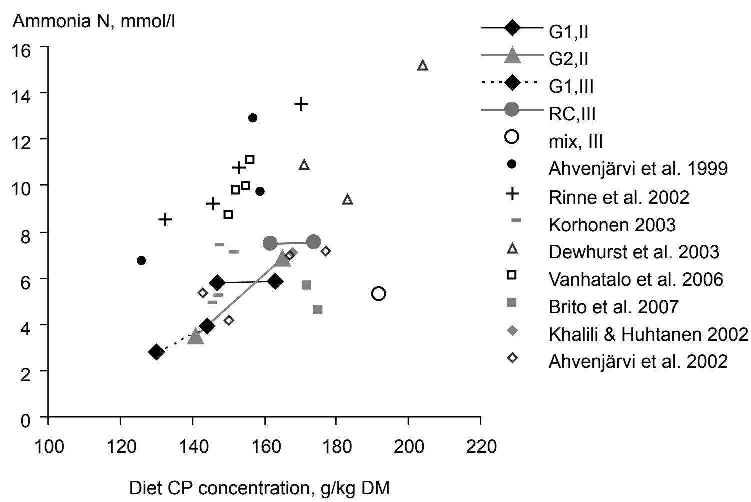


Figure 8. Concentration of ammonia N relative to diet CP concentration, in the rumens of dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2).

## 3.4 Nitrogen digestion and microbial protein synthesis in the rumen

In relation to dietary N concentration of diets, the average rumen ammonia N concentrations obtained in II and III may be considered relatively low, compared with previous data for grass silage diets (Ahvenjärvi et al. 1999, Korhonen 2003, Rinne et al. 2002) and for red clover diets (Dewhurst et al. 2003) (Figure 8). There might have been a slight deficiency of rumen degradable N for the diets based on the both primary growth grass silages of III and late harvested regrowth grass silage of II, as observed average ammonia N concentrations (3.92, 2.82 and 3.48 mmol/l for grass silages of III and II, respectively), and the minimum values during the feeding interval in the rumen of cows consuming them, were low.

The rumen ammonia N concentration for those diets was near the values, reported by Satter and Slyter (1974), required for optimal microbial protein synthesis (3.6 mmol/l) and was lower than the suggested minimum level for optimum fibre digestion (5.7 mmol/l; Hoover 1986). On the other hand, the observed average concentration of milk urea for late harvested regrowth grass diet (II) was higher than 16.0 mg/100 ml. It has been suggested that this value indicates an adequate supply of rumen degradable protein in the diet (Nousiainen 2004). However, in III, milk urea concentrations for both grass diets remained under that value, supporting the possible deficiency of rumen degradable N. Broderick et al. (2010) reported that zero ruminal N-balance (omasal CP flow = CP intake) was obtained at a dietary CP concentration of 147 g/kg DM, corresponding to a ruminal ammonia N of 5.1 mmol/l.

Omasal canal flow of microbial NAN increased consistently for all diets with increasing ME intake (Figure 9). Microbial protein synthesis is primarily connected to energy supply for rumen microbes.

### 3.4.1 Red clover vs grasses

Owing to the higher N concentrations, the N intakes were much higher for red clover than grass silage fed cows, being in the range of values obtained for pure red clover diets in other studies (Dewhurst et

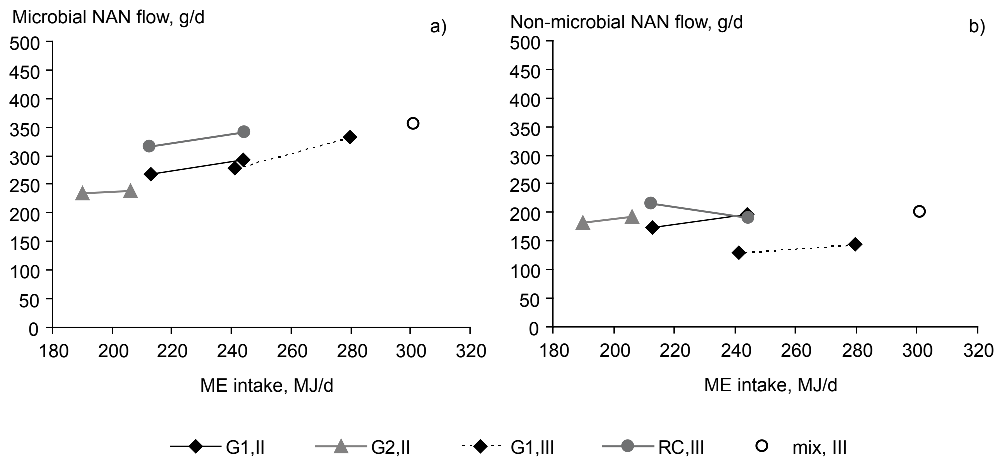


Figure 9. Omasal canal flow of microbial a) and non-microbial b) NAN of dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2).

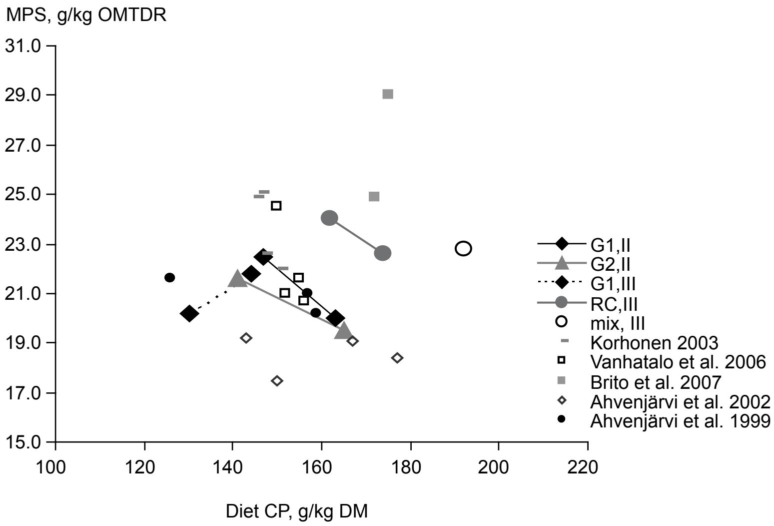


Figure 10. The efficiency of microbial protein synthesis (MPS, g/kg OM truly digested in the rumen (OMTDR)) in relation to diet CP concentration of dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2).

al. 2003b, Merry et al. 2006, Broderick et al. 2007).

The higher omasal canal NAN flow observed for red clover diets, compared with grass silage diets in III, was mainly composed of non-microbial NAN, due to lower N digestibility in the rumen with red clover diets. This is in agreement with previous results (Dewhurst et al. 2003a, Merry et al. 2006, Vanhatalo et al. 2006).

The efficiency of microbial protein synthesis (MPS) was higher for red clover diets, in agreement with previous results with dairy cows (Dewhurst et al. 2003a, Vanhatalo et al. 2006) but not with steers (Lee et al. 2003, Merry et al. 2006). The increase in the efficiency of MPS with advancing maturity was in agreement with Brito et al. (2007). Omasal canal NAN flow per DOM intake was higher for red clover diets than grass diets (40.2 vs 32.0 g NAN/ kg DOM intake).

For the mixed red clover grass silage diet, positive associative effects for intake were observed. One effect was an increased ammonia N concentration in the rumen for the mixed forage diet compared with the late-cut grass silage diet, which enhanced microbial synthesis. Higher omasal canal flow of total NAN and microbial NAN (Figure 9a) compared with both components of mixture and higher efficiency of MPS (Figure 10) compared with grass diets were observed in III.

### 3.4.2 Primary growth grass vs regrowth grass

The intake of N was higher for primary growth grass diets compared with regrowth grass diets (II), as well as for primary growth grass diets in II compared with those of III. Lower total NAN flow entering the lower tract with regrowth diets, compared with primary growth diets, was mainly explained by lower microbial NAN flow. The contribution of microbial NAN to the total NAN was, on average, lower for regrowth (56%) than for primary growth diets (60%). No difference between cuts was found in dietary NAN flow, in spite of the lower N digestibility in the rumen with regrowth diets. The average efficiency of microbial protein synthesis was not different between the cuts, therefore the lower NAN flow of regrowth diets was due to the lower DMI and lower amount of OM digested in the rumen.

Improved amino acid (AA) to ME balance at the tissue level could be a mechanism influencing intake as suggested by increased silage DMI in response to protein supplementation in dairy cows (Huhtanen et al. 2008). However, lower silage DMI for regrowth silage diets was most likely not due to lowered balance because higher ratio of metabolizable protein (MP) intake to ME intake was observed for regrowth grass diets compared with primary growth (8.1 vs 8.0 g MP/MJ ME for cut 2 and cut 1, respectively), in study II. Omasal canal NAN flow per digestible OM intake was also not affected by the cut.

The N intake for grass silage diets were lower in III compared with II due to a substantially lower N concentration of grass silages in III in spite of higher digestibility. The advancing maturity increased the efficiency of MPS, except for grass silage diets in III (Figure 9). In contrast, the efficiency of MPS decreased from early to late stage of growth, indicating an inadequate supply of N in the rumen for that late stage, as also indicated by low rumen ammonia N concentration and low milk urea concentration.

## 3.5 Milk yield and composition

### 3.5.1 Red clover vs grasses

Milk and ECM yields for red clover diets were similar to grass diets (III) despite the lower DMI. Red clover stimulates higher milk production irrespective of the variable intake responses compared with grasses, either fed alone or as a mixture with grasses (Thomas et al. 1985, Heikkilä et al. 1992, 1996, Randby 1992, Tuori et al. 2000, 2002, Dewhurst et al. 2003b, Bertilsson and Murphy 2003, Vanhatalo et al. 2006, Moorby et al. 2009). In Tables 5 and 6, the average milk and ECM yields were higher for red clover containing diets (+1.3 and +0.8 kg/d, for milk and ECM yields, respectively) or when it was fed as sole forage (+1.6 and +0.8 kg/d, for milk and ECM yields, respectively).

The higher milk production response may be caused by higher DMI or improved utilization of nutrients, or both. Higher silage DMI for red clover containing diets was also observed in Tables 5 and 6. However, with pure red clover diets the higher milk response was related to lower intake response, compared with diets where red clover was mixed with grass. It seems that red clover silage diets provide more nutrients with less feeds. Vanhatalo et al. (2006) concluded from an experiment where red clover grass silage (40% red clover) was compared with grass silage on the restricted feeding, that higher production responses cannot be explained only by increased DMI, but may be related to improved utilisation of nutrients.

In III, the ECM production remained unchanged in spite of the lower average ME intake, suggesting more efficient utilisation of ME for red clover diets compared with grass diets with average values of 0.121 vs 0.112 kg ECM/MJ ME, for red clover and grass diets, respectively (Figure 11, Table 9). This was in agreement with Moorby et al. (2009) who reported increased efficiency in terms of digestible DM intake with the increased proportion of red clover in diet.

The efficiency in III was also higher in terms of milk yield per kg of DM intake for red clover diets (1.32 vs 1.26 for red clover and grass diets, respectively, Table 9) which contrasted with the results of Moorby et al. (2009). They suggested that the increased efficiency indicated mobilization and use of body energy reserves for milk production for red clover silage diets. Higher plasma NEFA concentrations on red clover, than on grass diets, may indicate increased use of body reserves also in III. Improvement of nutrient utilisation was supported by the higher OM and pdNDF digestibility for red clover diets compared with grass diets (IV).

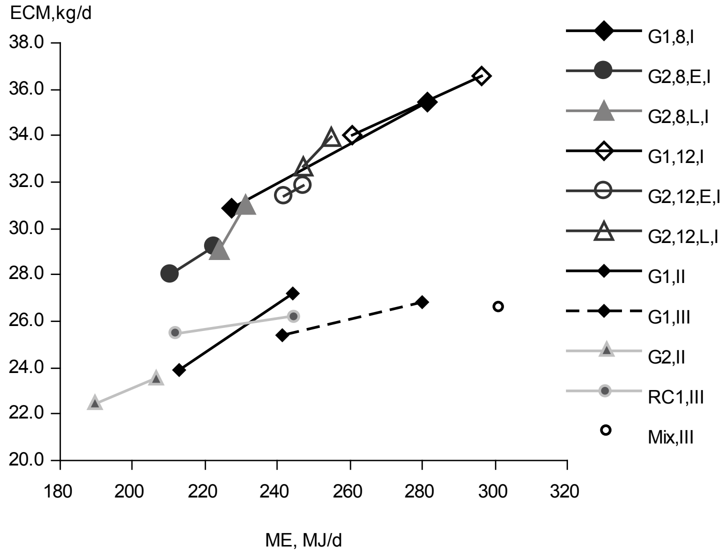


Figure 11. The effect of intake of metabolizable energy (ME) on energy corrected milk (ECM) yield of dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and grass silages from regrowth (2) in studies I, II and III. In I, two levels of concentrates (8 and 12 kg/d) were offered.

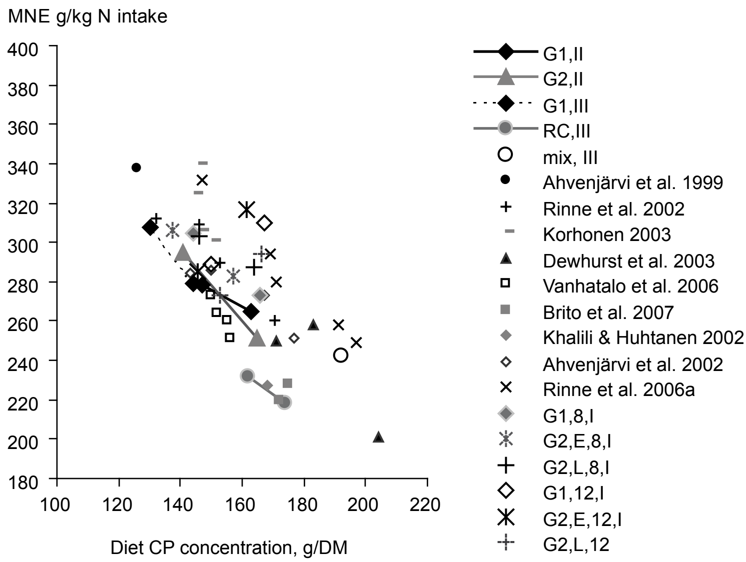


Figure 12. Milk N efficiency (MNE, milk N, g/kg N intake) in relation to diet CP concentration of dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) and from regrowth (2) or mixed red clover-grass silages.

Table 9. The milk N and energy efficiency of dairy cows consuming diets based on grass (G) and red clover (RC) silages from primary growth (1) or from regrowth (2), harvested at early (E) or late (L) stage of maturity. Concentrate levels were 8 and 12 kg/d in I, 8 kg/d in II and 9 kg/d in III, IV.

| | MNE[1], g/kg N intake | ECM, kg/kg DMI | ECM, kg/MJ ME |
|---|---|---|---|
| **I** | | | |
| G1,E,8 | 273 | 1.46 | 0.126 |
| G1,L,8 | 305 | 1.50 | 0.124 |
| G2,EE,8 | 283 | 1.47 | 0.136 |
| G2,EL,8 | 306 | 1.43 | 0.130 |
| G2,LE,8 | 287 | 1.51 | 0.132 |
| G2,LL,8 | 303 | 1.41 | 0.129 |
| G1,E,12 | 310 | 1.45 | 0.134 |
| G1,L,12 | 289 | 1.46 | 0.130 |
| G2,EE,12 | 317 | 1.47 | 0.135 |
| G2,EL,12 | 285 | 1.43 | 0.134 |
| G2,LE,12 | 294 | 1.53 | 0.131 |
| G2,LL,12 | 273 | 1.47 | 0.132 |
| *Cut*[2] | *o* | *NS* | * |
| *Maturity* | *** | *NS* | *** (cut 1), NS (cut 2)* |
| **II** | | | |
| G1,E | 265 | 1.31 | 0.112 |
| G1,L | 278 | 1.24 | 0.112 |
| G2,E | 251 | 1.29 | 0.114 |
| G2,L | 295 | 1.28 | 0.118 |
| *Cut* | *NS* | *NS* | *NS* |
| *Maturity* | * | *NS* | *NS* |
| **III, IV** | | | |
| G1,E | 279 | 1.26 | 0.110 |
| G1,L | 308 | 1.27 | 0.114 |
| Mix | 243 | 1.24 | 0.110 |
| RC,E | 218 | 1.34 | 0.119 |
| RC,L | 232 | 1.30 | 0.122 |
| *G vs RC* | *** | * | ** |
| *Maturity* | * | *NS* | *NS* |

[1]MNE = efficiency of N for milk protein synthesis (milk N produced / N intake). [2]Statistical significance within the trial *o* $P \leq 0.10$, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, NS = not significant ($P > 0.05$).

Lower milk fat and protein concentrations for red clover diets in III compared with grass silage diets were in agreement with earlier reports (Tables 5 and 6), whereas they were unchanged in one study reported by Bertilsson and Murphy (2003) and in the study of Dewhurst et al. (2003b).

The AA supply of cows fed red clover versus grass silage diets was higher (III). However, milk protein content and yield of red clover-fed cows did not reflect the better supply, suggesting a possible imbalance in nutrients available for milk synthesis. Cows consumed ME and MP in excess of the requirements according to MTT (2006), suggesting no lack of MP quantitatively. It can be assumed that the imbalance in AA due to inadequate Met supply could have limited further production responses, especially for milk protein. This was supported by recent results where pure red clover (Vanhatalo et al. 2009) or mixed red clover grass diets (Rinne et al. 2006a) were supplemented with rapeseed or soya-bean expeller. The concentration of Met in plasma and protein content in milk increased with increasing amount of rapeseed expeller in the diet (Vanhatalo et al. 2009). Rapeseed expeller contain more Met compared with soya-bean expeller, and may also contain more rumen-undegradable protein (MTT 2006).

The efficiency of N utilization for milk protein synthesis (MNE, ratio of milk N produced to N intake) was lower for red clover diets than for grass diets but seems to be negatively related to diet CP concentration in a similar way as for grass silage diets (Figure 12).

### 3.5.2 Primary growth vs regrowth

Milk and ECM yields, and fat and protein concentrations, were lower when cows consumed regrowth grass silages compared with primary growth grass silages (I), which was in agreement with the data presented in Table 7. The mean responses of cows to regrowth grass silage in terms of silage DMI and ECM yield were -1.8 kg/d and -2.7 kg/d, respectively. This was to be expected because the D-value of regrowth silage was lower both in the study I and also in Table 7.

The higher milk yield observed for primary growth grass diets may be caused by higher DMI and subsequently higher ME intake, or improved utilization of ME, or both. In I and II, the ECM production was closely related to ME intake and no evidence of better utilization of ME for primary growth grass diets was found (Figure 11, Table 9). That is in agreement with Peoples and Gordon (1989) and Heikkilä et al. (1998) who compared spring and autumn grass silages and concluded that the lower milk production potential of autumn silages was due to reduced silage DMI with no difference in the efficiency of energy utilization.

The average MNE was 272 g/kg in II (Table 9). The difference between cuts was not significant, although the lowest value was observed for the early harvested regrowth silage diet, where the milk urea concentration was also high. However, MNE was not different for that diet in relation to diet CP concentration compared with other diets in II or III (Figure 12).

The response of milk yield to maturity was smaller for regrowth grass silage diets compared with primary growth, with average values of 0.42 and 0.21 kg per 10 g decline in D-value for primary growth and regrowth, respectively (I). This is in agreement with the results of Rinne (2000) who reported 0.3–0.5 kg response per 10 g of D-value for primary growth grass silages.

# 4 Concluding remarks

1. Red clover contained more ash, protein and lignin and less NDF than grasses. The concentration of iNDF was higher compared with grass, causing a lower pdNDF concentration and a higher ratio of iNDF to NDF. In all data (own results and literature review), the mean ratio was 0.350 for red clover and 0.180 for grasses.

2. Regrowth grass contained more ash, protein, iNDF and less NDF than grass from primary growth. Higher iNDF concentration with lower NDF concentration induced higher ratio of iNDF to NDF, 0.193 for regrowth and 0.167 and for primary growth grass, in all data obtained.

3. In regrowth grass, the proportion of leaves at comparable D-value was higher than in primary growth. Furthermore, higher proportions of other plant species (weeds) and dead tissues, and a higher proportion of meadow fescue than timothy were found in regrowth compared with primary growth.

4. In primary growth, the mean daily decrease of digestibility was slower for red clover than for grasses (5.1 vs 3.7 g/d for grass and red clover, respectively). The mean daily rate of increase in NDF concentration was higher for grasses (4.6 vs 4.3 g/d for grasses and red clover, respectively) whereas the rate of iNDF increase was higher for red clover (3.7 vs 4.1 g/d for grasses and red clover, respectively).

5. The mean daily decrease of digestibility was clearly slower for regrowth grass compared with primary growth grass (1.4 vs 5.1 g/d, respectively). Also the mean daily rate of increase in NDF and iNDF concentration was slower for regrowth grass compared with primary growth grass (0.8 vs 4.6 g/d for NDF and 1.2 vs 3.7 g/d for iNDF concentrations, respectively).

6. In regrowth, the average digestibility decreased with delayed harvest by a slightly higher rate for red clover than for grasses (1.6 vs 1.4 g/d, respectively). The average daily increase in NDF was considerably higher for red clover (3.2 g/d) than for grasses (0.8 g/d), and that of iNDF was also much higher for red clover (4.0 g/d) than for grasses (1.2 g/d).

7. Intake and milk production were slightly lower at comparable digestibility when diets were based on regrowth rather than primary growth grass silages. Lower milk production responses to regrowth grass silage diets could not be accounted for differences in energy or protein utilization, but were mainly due to the lower silage DMI.

8. Regrowth grass silage intake was not limited by NDF digestion, rumen fill or passage kinetics. Instead, the lower intake may be at least partly related to plant diseases such as leaf spot infections, dead material or occurrence of weeds, which all were more abundant in regrowth than in primary growth grass. More attention should be paid to this since the importance of regrowth silages as a forage supply in Northern areas may possibly increase owing to predicted global warming in the future.

9. The change in digestibility of primary growth and regrowth grass silages affected differently intake and milk production of cows. The effect of delayed harvest was less pronounced in regrowth than in primary growth. However, no firm conclusions could be drawn due to the confounding effect of variable DM contents

of silages, limited amount of data and relatively small range in silage quality.

10. The intake and milk production responses to red clover silage diets were higher compared with grass silage diets. Over all data, the silage DMI was on average 0.4 – 1.3 kg/d higher and ECM yield 0.8 kg/d higher for red clover diets compared with grass diets.

11. The efficiency of energy use for milk production in terms of kg ECM yield per kg feed DMI was higher for red clover compared with grass silage diets. The milk N efficiency was lower for red clover than for grass silage diets, reflecting a higher N intake with red clover diets. Further milk production responses to red clover might have been limited by the imbalance of AA supply, especially inadequacy of methionine.

12. Intake and production were differently affected by changes in digestibility when the silages were prepared from red clover, compared with grass silages. The D-value did not explain the intake and production potential of red clover silage as consistently as it did for grass silages.

13. The response to concentrate inclusion was not similar for regrowth grass silage diets compared with primary growth silages diets. The ECM response to increased concentrate allowance was greater (0.92 vs 0.62 kg/kg concentrate) when regrowth, rather than primary growth, grass silage diets were fed. The ECM response increased with increasing maturity in both cuts.

14. Further research should be directed especially towards elucidating the effects of growth stage of red clover and regrowth grass on silage DMI and milk production.

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